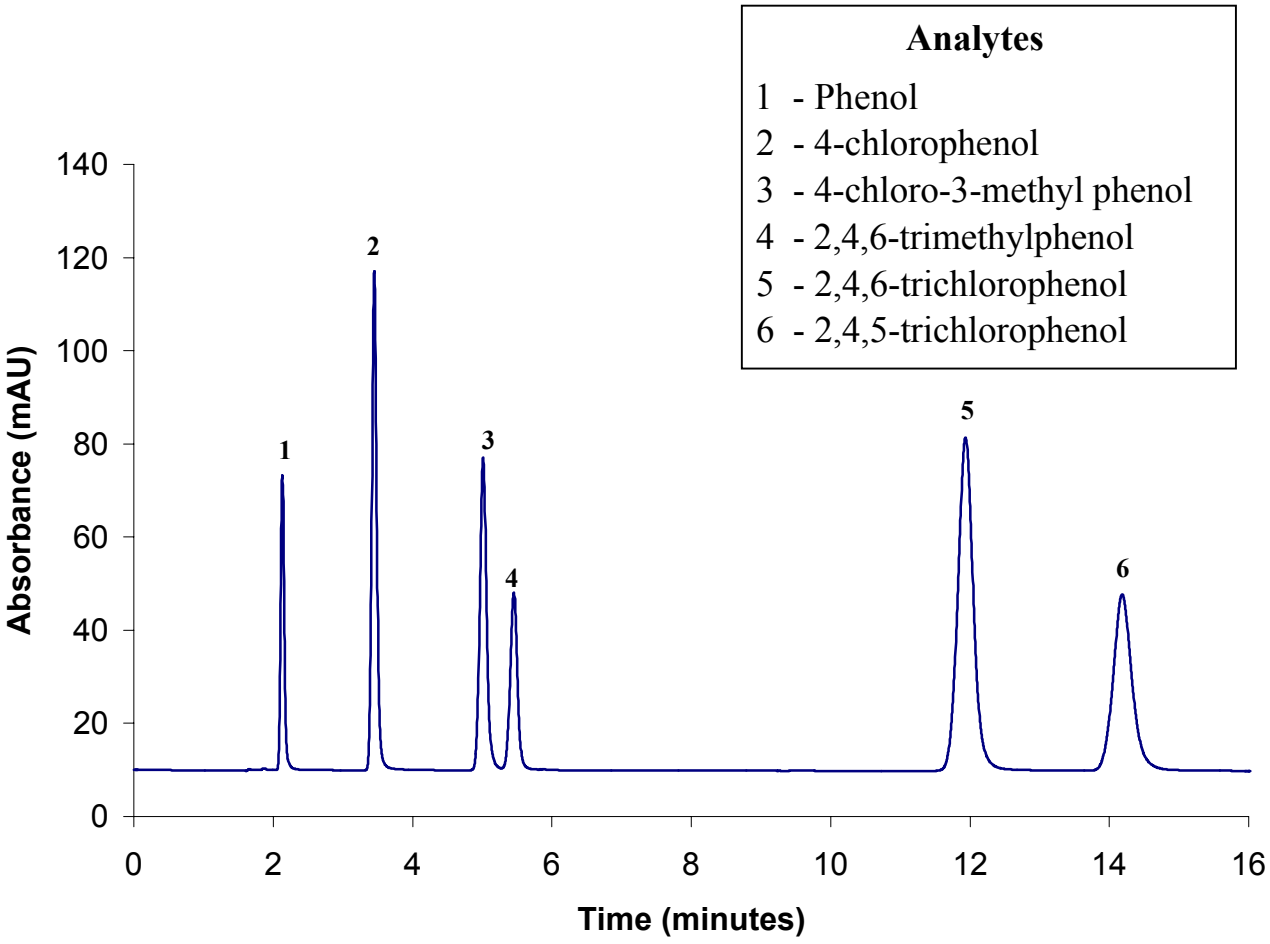
ZirChrom®

Technical Bulletin #131

... For Peak Performance

Chlorophenols Separation on ZirChrom®-PBD



LC Conditions

Column: ZirChrom®-PBD, 150 mm × 4.6 mm i.d.
Mobile Phase: 25/75 A/B
A: Acetonitrile
B: 20mM ammonium acetate and 20 mM sodium fluoride, pH 4.10

Flow rate: 1.0 mL/min.
Temperature: 30 ºC
Detection: 280 nm
Pressure Drop: 141 bar

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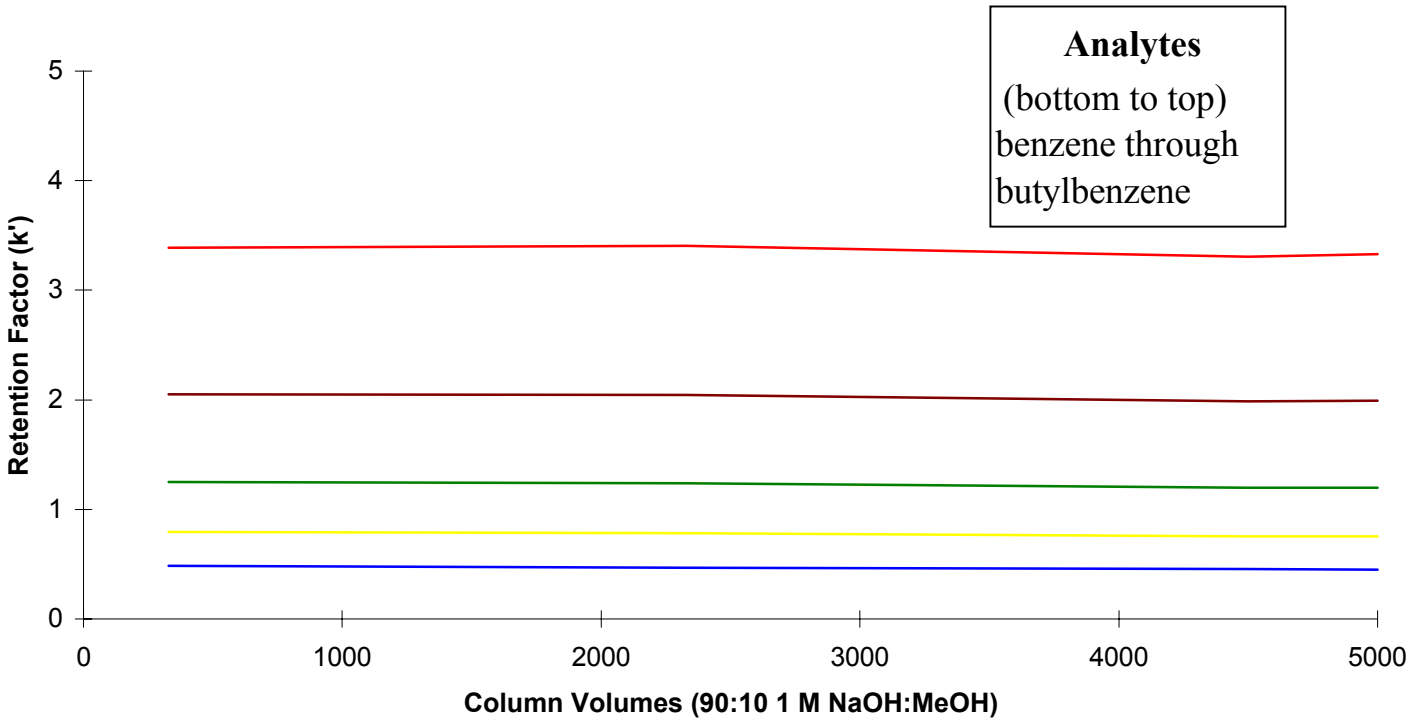
Technical Bulletin #132

... For Peak Performance

ZirChrom®-PBD Stability After Base Wash



LC Conditions

Column: ZirChrom®-PBD, 150 × 4.6 mm

Mobile Phase: 50/50 A/B Wash Fluid: 90/10 C/D

A: Acetonitrile B: Water

C: 1M sodium hydroxide in water D: Methanol

Flow rate: 1.0 mL/min.

Temperature: 35 ºC

Detection: 280 nm

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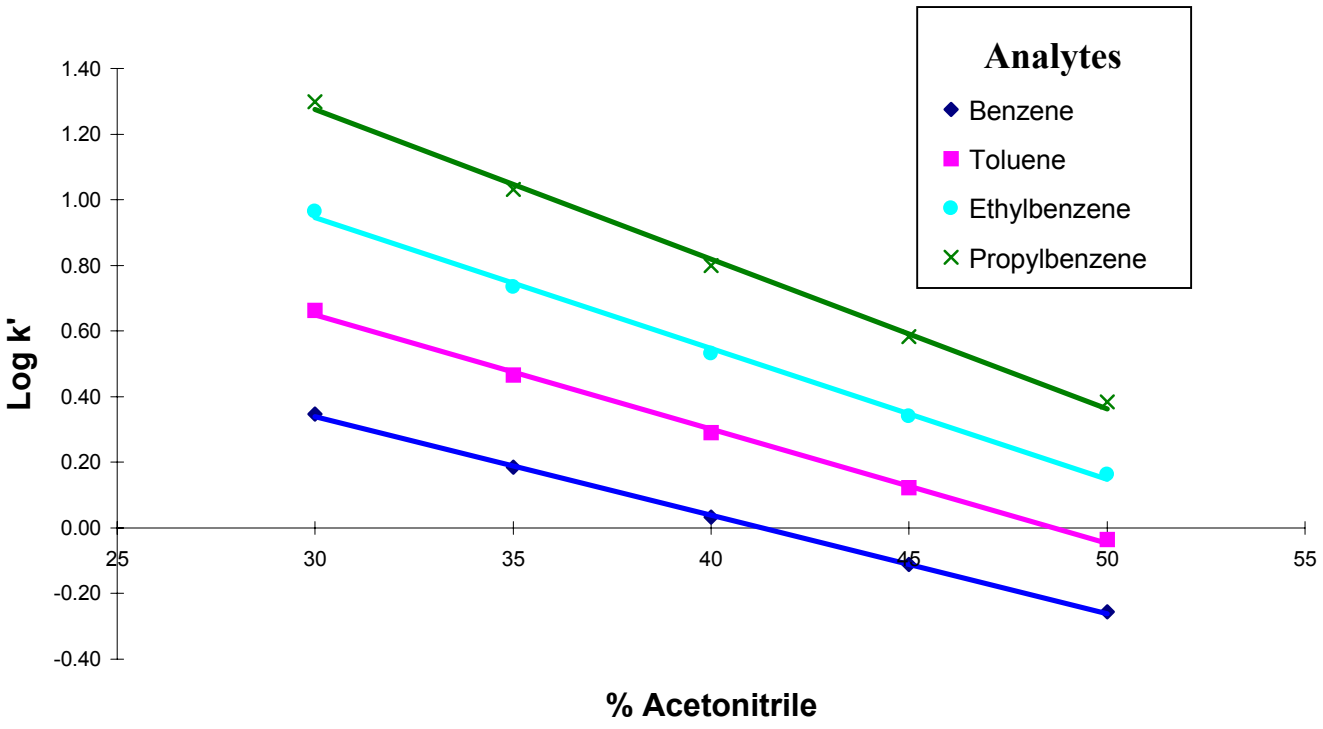
Technical
Bulletin #133

... For Peak Performance

Reversed-Phase Retention Behavior of ZirChrom®-PBD



LC Conditions

Column: ZirChrom®-PBD, 150 × 4.6 mm
Mobile Phase: A/B
A: Acetonitrile
B: Water

Flow rate: 1.0 mL/min.
Temperature: 30 ºC
Detection: 280 nm

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Technical Bulletin #172

... For Peak Performance

ZirChrom®-PBD Outperforms Polymeric Columns

ZirChrom®-PBD columns

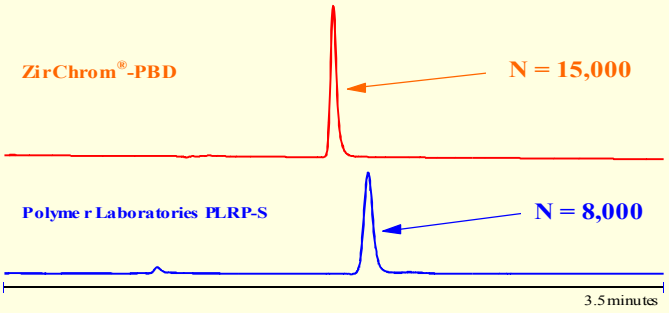
vs.

Polymeric columns

ZirChrom®-PBD columns offer a superior reversed-phase alternative to polymeric columns. ZirChrom®-PBD columns possess all of the advantages of polymeric columns with none of the drawbacks. Like polymeric columns, ZirChrom®-PBD columns offer extreme chemical and thermal stability. Unlike polymeric columns, ZirChrom®-PBD columns possess superior efficiency, are highly reproducible (particularly for gradient elutions), exhibit selectivity similar to bonded phases for easier method development, and offer extraordinary solvent stability. Try a ZirChrom®-PBD column today! If you are not completely satisfied with its performance just return the column within ninety days for a full money back refund.

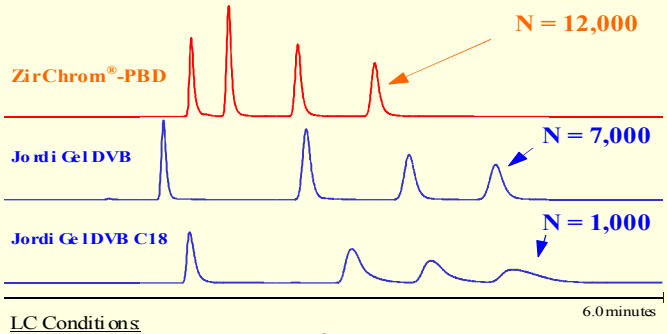
ZirChrom® columns - *Most Novel for 1998 by LC•GC*

Figure 1. Comparison of ZirChrom®-PBD to Polymer Labs PLRP-S 100A using Polymer Labs reversed-phase test mix and test conditions.



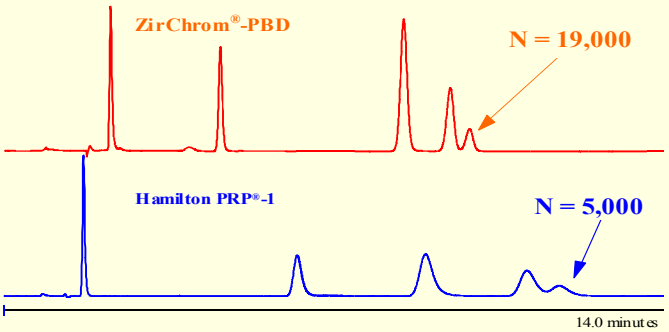
LC Conditions:
Columns (top to bottom), ZirChrom®-PBD, Polymer Laboratories PLRP-S 100A, 150 x 4.6 mm i.d.; Mobile Phase, 87.5/12.5 Acetonitrile/Water; Flow, 1.0 ml/min; Detector, 254 nm; Column Temperature = 30°C; Injection Volume, 0.5 µl; Solute: phenol.

Figure 2. Comparison of ZirChrom®-PBD to Jordi Gel DVB 500A and DVB C18 500A using Jordi reversed-phase test mix and test conditions.



LC Conditions:
Columns (top to bottom), ZirChrom®-PBD, Jordi Gel DVB, Jordi Gel DVB C18 500A, 150 x 4.6 mm i.d.; Mobile Phase, 10(10)/10(20)/20(30)/60(40) Methanol/Acetonitrile/Tetrahydrofuran/Water; Flow, 1.0 ml/min; Detector, 254 nm; Column Temperature = 50°C; Injection Volume, 2.0 µl; Solutes: uracil, ethylparaben, propylparaben, butylparaben.

Figure 3. Comparison of ZirChrom®-PBD to Hamilton PRP®-1 using ZirChrom®-PBD reversed-phase test mix and test conditions.



LC Conditions:
Columns (top to bottom), ZirChrom®-PBD, Hamilton PRP®-1, 150 x 4.6 mm i.d., Mobile Phase, 15(50)/85(50) Acetonitrile/Water; Flow, 1.0 ml/min; Detector, 254 nm; Column Temperature = 30°C; Injection Volume, 5.0 µl; Solutes: resorcinol, benzonitrile, methyl benzoate, anisole, benzene.

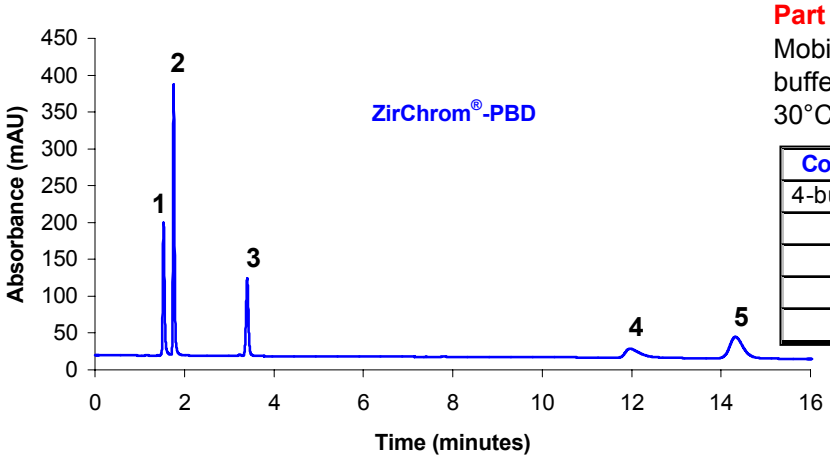
ZirChrom®

Technical Bulletin #174

... For Peak Performance

ZirChrom®-PBD is the clear choice over Waters Symmetry® for basic compounds.

ZirChrom®-PBD columns offer a stable reversed-phase alternative to Waters Symmetry® columns for extreme chemical and thermal stability, while maintaining high column efficiency and excellent peak symmetry.



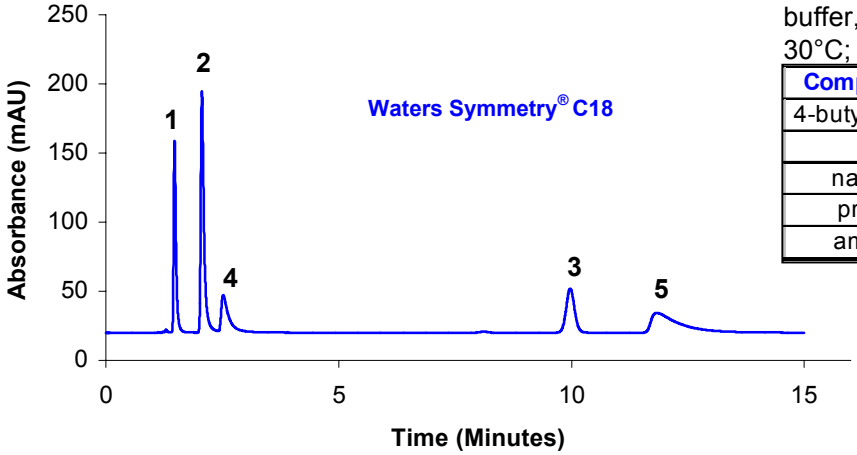
ZirChrom®-PBD (3 µm) 150 x 4.6 mm
Part #ZR03-1546

Mobile phase: 55/45 Acetonitrile/20 mM phosphate buffer, pH=7.0; Flow rate: 1.0 ml/min; Temperature: 30°C; Detector wavelength: 254 nm.

Compound Name	k'	Plates	Symmetry
4-butyl benzoic acid	0.00	9,000	0.71
pyridine	0.15	14,900	0.77
naphthalene	1.22	20,000	0.91
propranolol	6.80	7,000	0.46
amitryptyline	8.34	11,250	0.71

Analytes

1 - 4-butyl benzoic 2 - pyridine 3 - napthalene
4 - propranolol 5 - amitryptyline



Waters Symmetry® C18 (3.5 µm) 150 x 4.6 mm

Mobile Phase: 55/45 Acetonitrile/20 mM phosphate buffer, pH=7.0; Flow rate: 1.0 ml/min; Temperature: 30°C; Detector wavelength: 254 nm.

Compound Name	k'	Plates	Symmetry
4-butyl benzoic acid	0.00	5,000	0.57
pyridine	0.40	5,500	0.51
naphthalene	5.76	15,400	0.92
propranolol	0.71	1,600	0.35
amitryptyline	7.02	2,350	0.24

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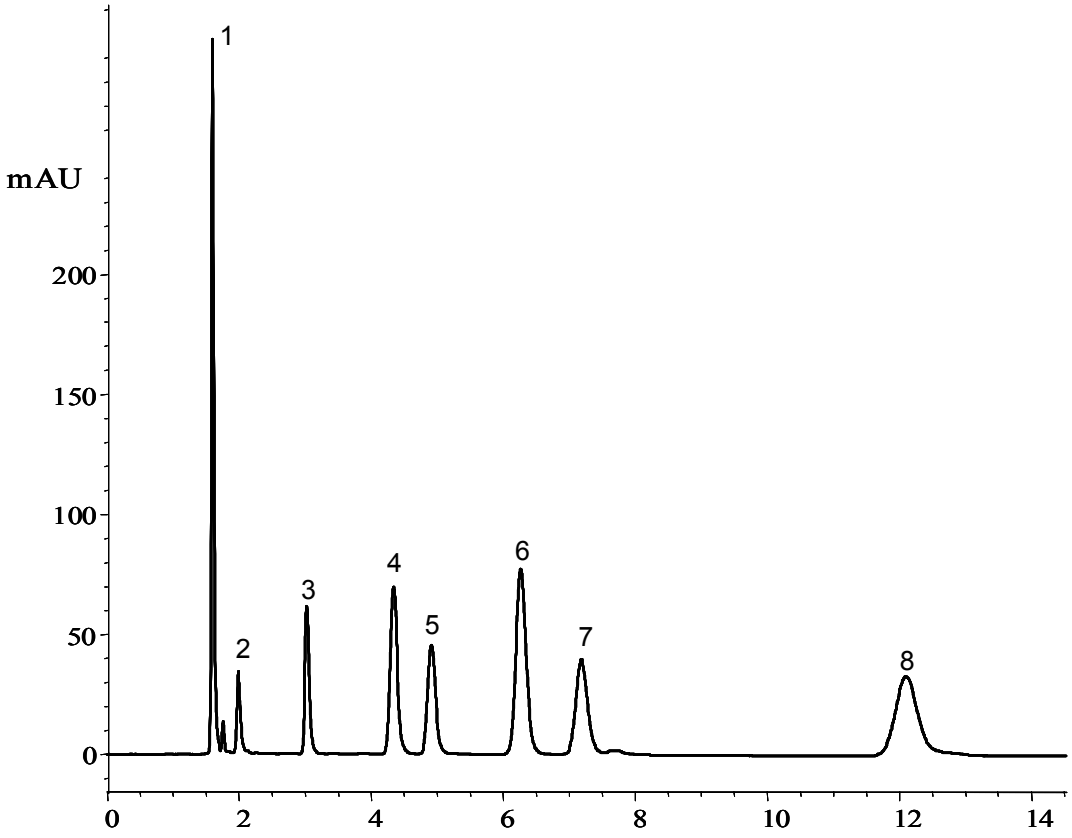
Technical Bulletin #198

... For Peak Performance

Opioids on ZirChrom®-PBD

Analytes

1 - Aminophylline
2 - Morphine
3 - Phenacetin
4 - Codeine
5 - Hydrocodone
6 - Ethylmorphine
7 - Oxycodone
8 - Papaverine



LC Conditions

Column: ZirChrom®-PBD, 150 × 4.6 mm
Mobile Phase: 15/85 A/B
A: Acetonitrile
B: 50 mM potassium phosphate monobasic at pH 10.0

Flow rate: 1.0 mL/min.
Temperature: 30 ºC
Detection: 220 nm

ZirChrom®

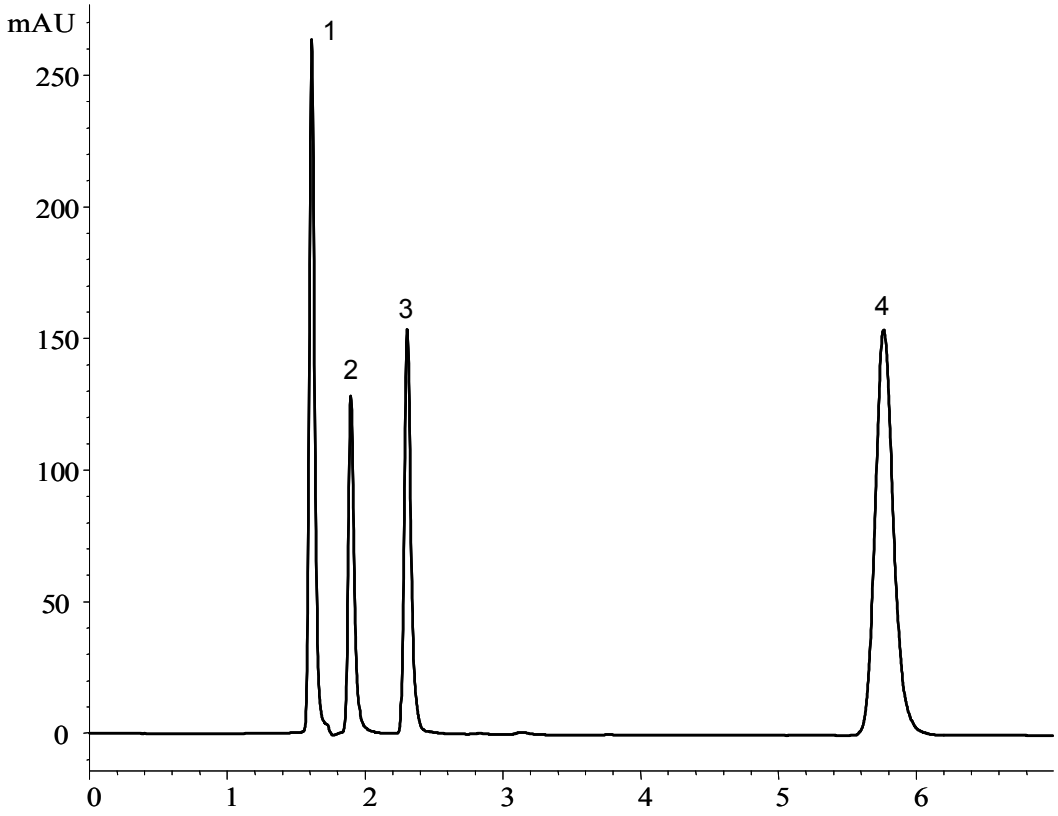
Technical
Bulletin #199

... For Peak Performance

Anticonvulsants on ZirChrom®-PBD

Analytes

1 - Phenobarbital
2 - Primidone
3 - Methsuximide
4 - Diazepam



LC Conditions

Column: ZirChrom®-PBD, 150 × 4.6 mm
Mobile Phase: 30/70 A/B
A: Acetonitrile
B: 50 mM potassium phosphate monobasic at pH 10.0

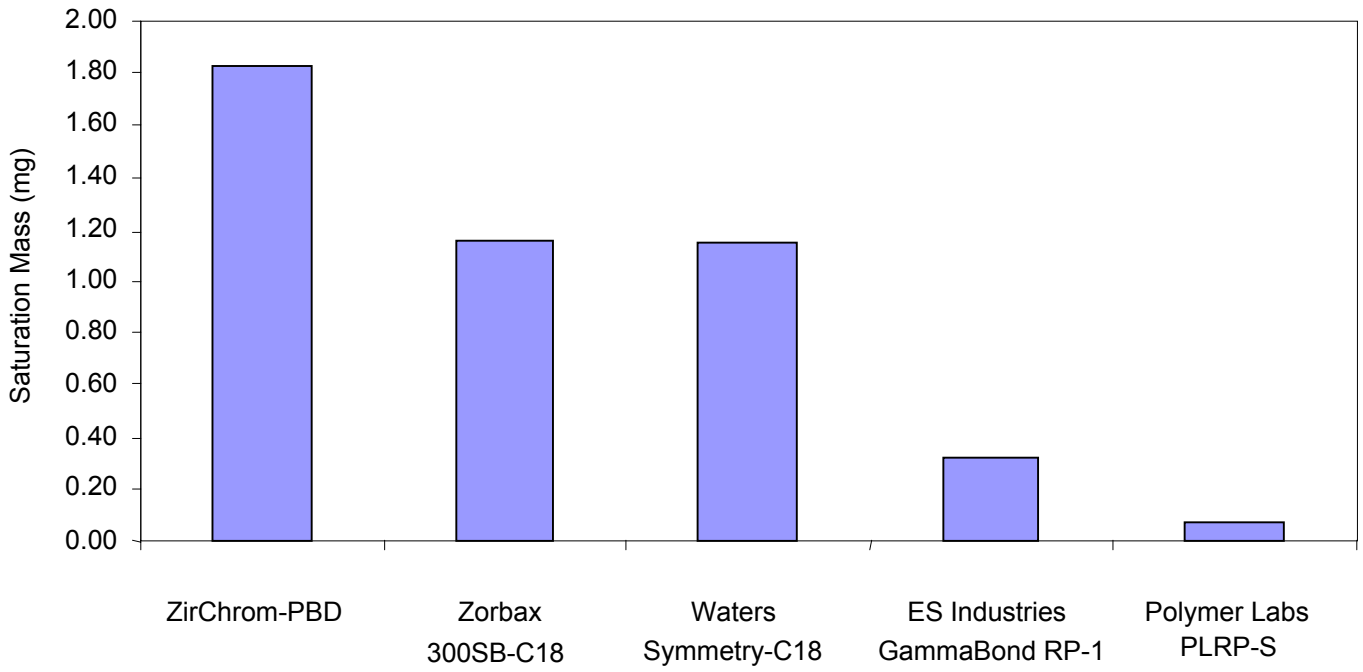
Flow rate: 1.0 mL/min.
Temperature: 30 ºC
Detection: 220 nm

ZirChrom®

Technical
Bulletin #200

... For Peak Performance

High Sample Loadability on ZirChrom®-PBD



ZirChrom®-PBD has excellent sample loadability for basic drugs compared to other "stable" reversed-phase columns. The columns studied were of the same dimensions (150 mm x 4.6 mm i.d.) and packed with stationary phases with similar specifications as ZirChrom®-PBD (3µm particle size, 300Å pore size). The basic drug used for this study was nortriptyline. Its retention factor was kept constant across all columns by adjusting the strength of the mobile phase. Saturation mass was calculated by the method used by Snyder and co-workers (L.R. Snyder, J.J. Kirkland and J.L. Glach, Practical HPLC Method Development (John Wiley & Sons, New York, 1997).

ZirChrom®

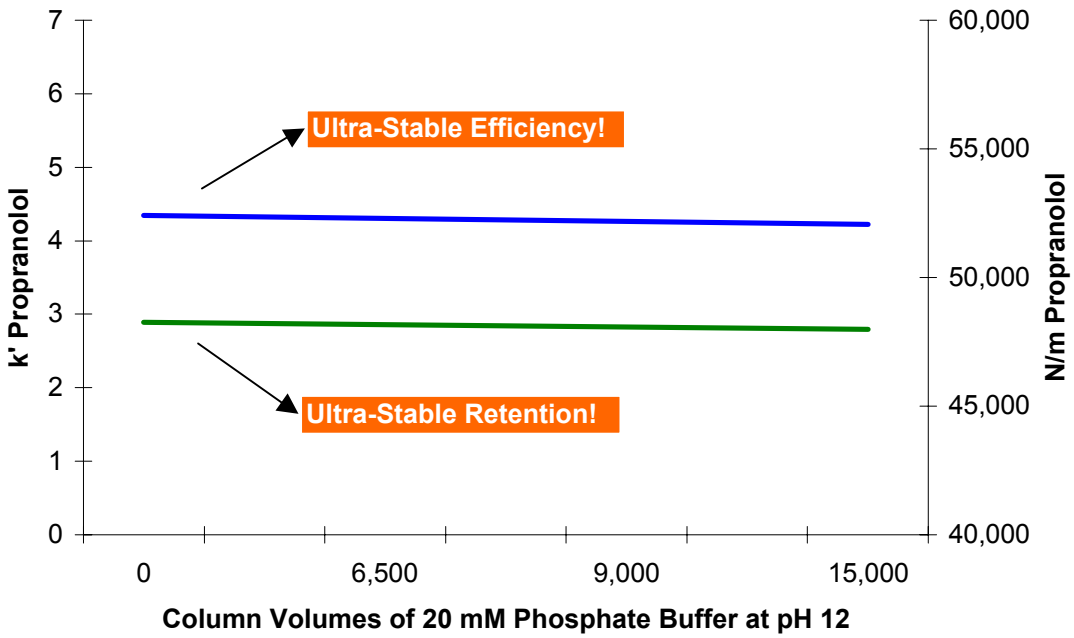
Technical Bulletin #201

... For Peak Performance

ZirChrom®-PBD: Ultra-Stable for Basic Drugs at pH 12

Analytes

1 - Labetalol
2 - Atenolol
3 - Acebutolol
4 - Metoprolol
5 - Oxprenolol
6 - Lidocaine
7 - Quinidine
8 - Alprenolol
9 - Propranolol



LC Conditions

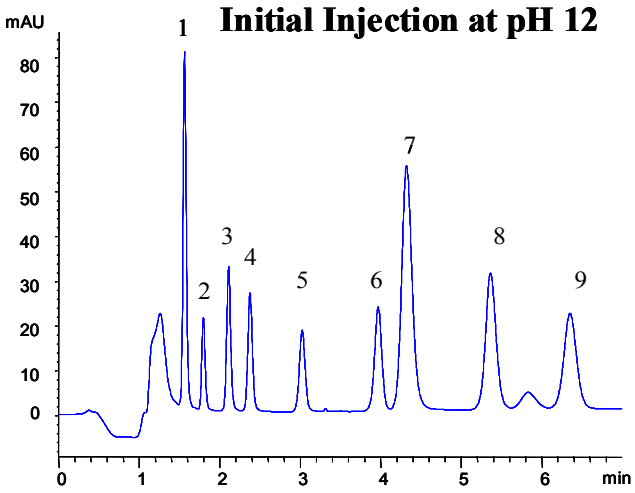
Column: ZirChrom®-PBD
Mobile Phase: 28/72 A/B
A: Acetonitrile
B: 20 mM potassium phosphate at pH=12.0

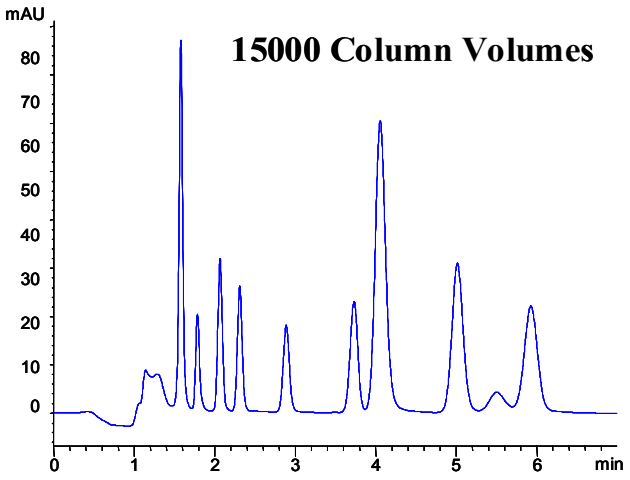
Flow rate: 1.0 mL/min.
Temperature: 30 °C
Detection: 254 nm

ZirChrom Separations, Inc.
Toll Free: (866) STABLE-1
Fax: (763) 421-2319
http://www.zirchrom.com

Column Life Comparison for ß Blockers

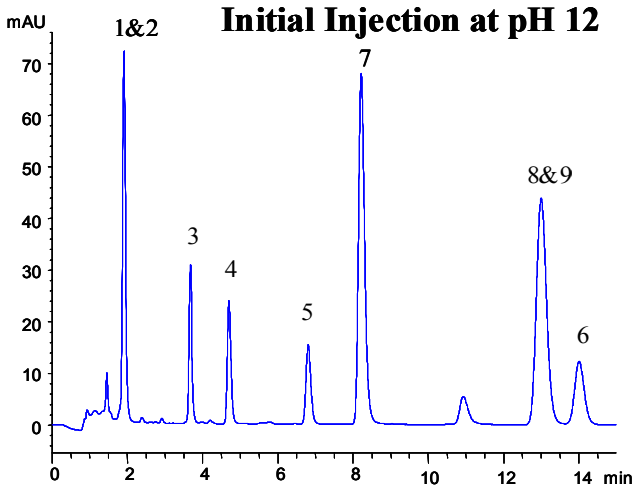
ZirChrom®-PBD

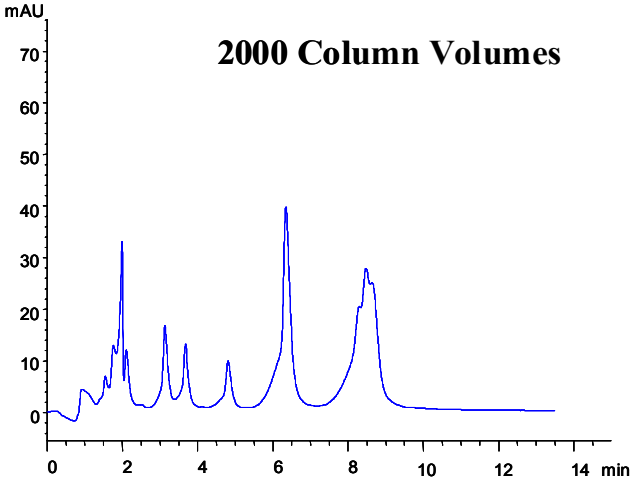




LC Conditions:
ZirChrom®-PBD; Mobile Phase, 28/72 acetonitrile/ 20 mM potassium phosphate at pH=12.0; Flow Rate, 1.0 mL/min.; Temperature, 30°C; Detection, 254 nm.
Solutes: 1=Labetalol, 2=Atenolol, 3=Acebutolol, 4=Metoprolol, 5=Oxprenolol, 6=Lidocaine, 7=Quinidine, 8=Alprenolol, 9=Propranolol.

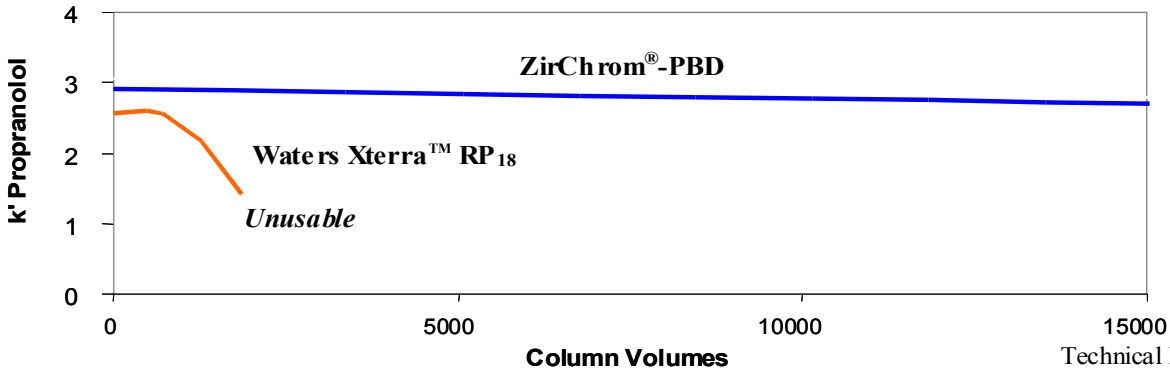
Waters Xterra™ RP$_{18}$





LC Conditions:
Waters Xterra™ RP$_{18}$; Mobile Phase, 35/65 acetonitrile/ 20 mM potassium phosphate at pH=12.0; Flow Rate, 1.0 mL/min.; Temperature, 30°C; Detection, 254 nm.
Solutes: 1=Labetalol, 2=Atenolol, 3=Acebutolol, 4=Metoprolol, 5=Oxprenolol, 6=Lidocaine, 7=Quinidine, 8=Alprenolol, 9=Propranolol.

Column Life Comparison at pH 12



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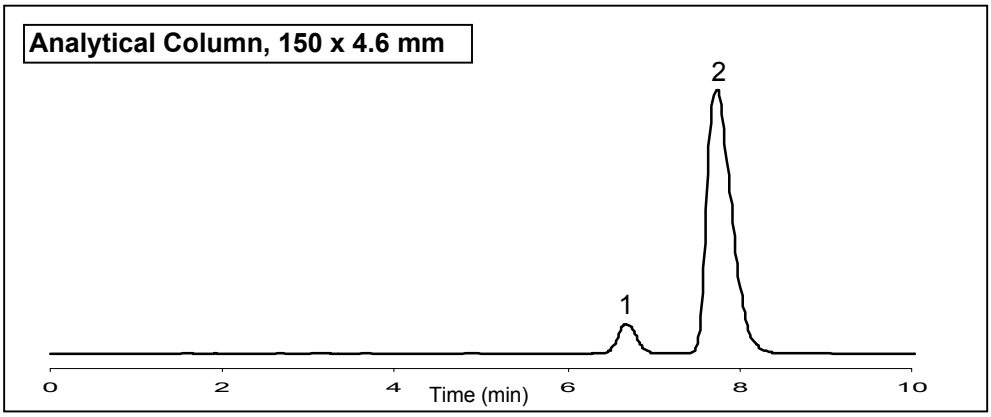
Technical Bulletin #210

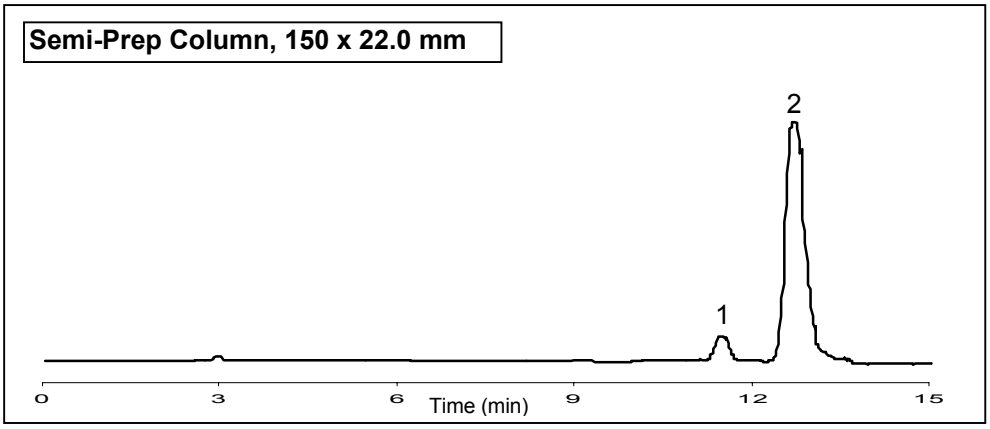
... For Peak Performance

Emamectic Separation and Scale-up on ZirChrom®-PBD

Analytes

1 - Emamectin B1a
2 - Emamectin B1b





LC Conditions

Column: ZirChrom®-PBD
Mobile Phase: 60/40 A/B
A: ACN
B: 50mM Ammonium Phosphate pH 3.5

Flow rate: 1.0 mL/min.
Temperature: ambient
Detection: 245 nm

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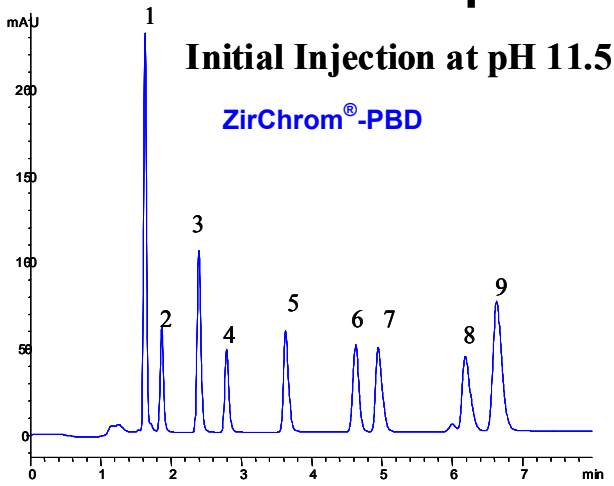
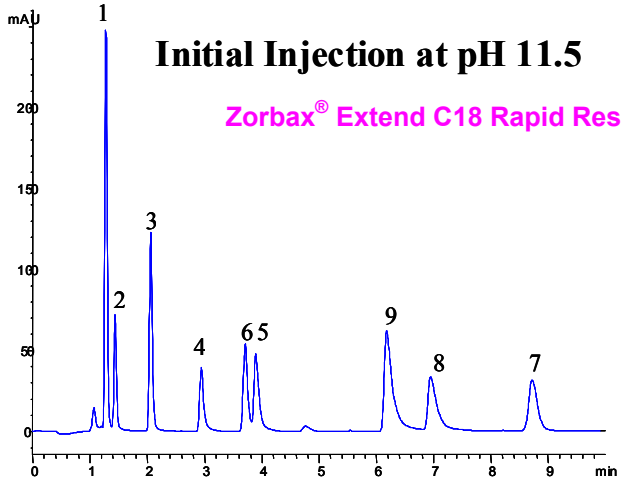
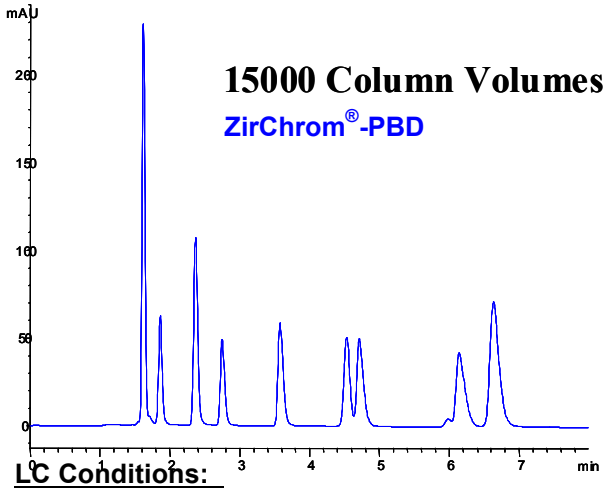
Technical Bulletin #212

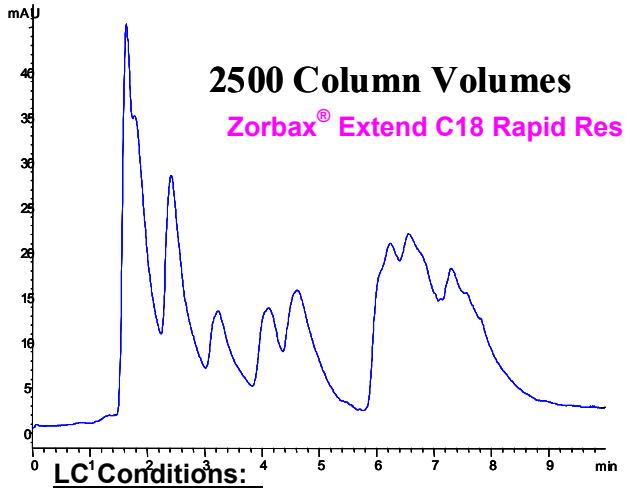
... For Peak Performance

Column Life Comparison for ß Blockers









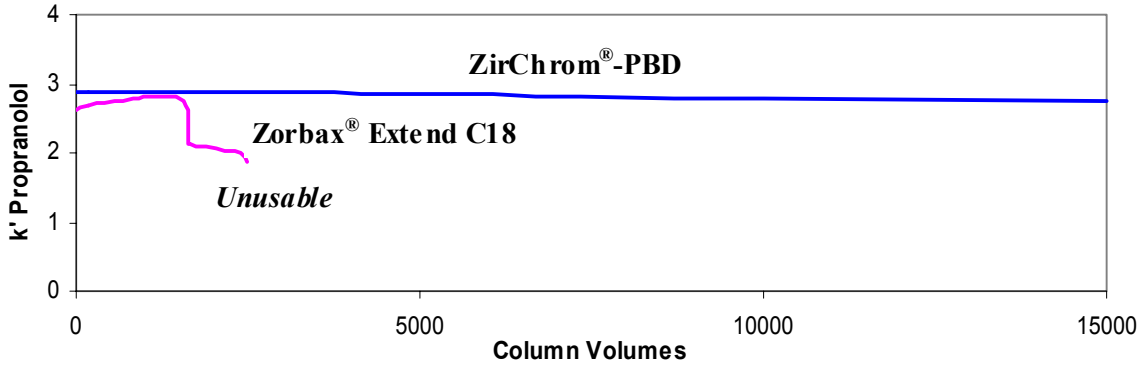
LC Conditions:

ZirChrom®-PBD; Mobile Phase, 28/72 acetonitrile/ 20 mM potassium phosphate at pH=11.5; Flow Rate, 1.0 mL/min.; Temperature, 40oC; Detection, 254 nm. Solutes: 1=Labetalol, 2=Atenolol, 3=Acebutolol, 4=Metoprolol, 5=Oxprenolol, 6=Lidocaine, 7=Quinidine, 8=Alprenolol, 9=Propranolol.

LC Conditions:

Zorbax® Extend C18; Mobile Phase, 45/55 acetonitrile/ 20 mM potassium phosphate at pH=11.5; Flow Rate, 1.0 mL/min.; Temperature, 40oC; Detection, 254 nm. Solutes: 1=Labetalol, 2=Atenolol, 3=Acebutolol, 4=Metoprolol, 5=Oxprenolol, 6=Lidocaine, 7=Quinidine, 8=Alprenolol, 9=Propranolol.

Column Life at pH 11.5

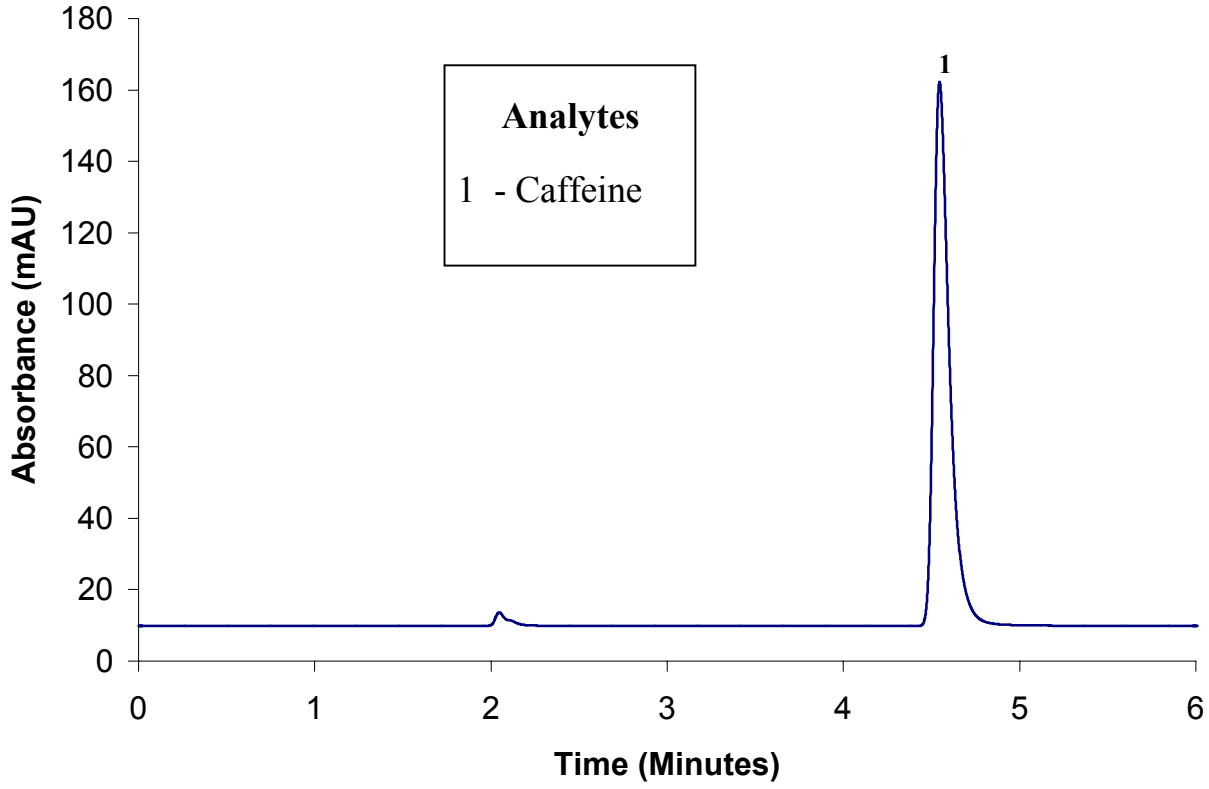


ZirChrom®

Technical
Bulletin #216

... For Peak Performance

Caffeine in Soda Analysis on ZirChrom®-PBD



LC Conditions

Column: ZirChrom®-PBD, 150 mm x 4.6 mm i.d.
Mobile Phase: 100% water
Pressure Drop: 102 bar

Flow rate: 1.0 mL/min.
Temperature: 50°C
Detection: 254 nm

ZIRCHROM SEPARATIONS, INC. - Manufacturers of Zirconia-Based HPLC Media.
Specialists in Ultra-Durable, High Efficiency HPLC Phases

Technical Bulletin #239

Recommendations for the Application of a Quantitative Assay for Azithromycin Using a ZirChrom®-PBD Column

HPLC Column

- A 15 cm x 4.6 mm i.d. ZirChrom®-PBD (part # ZR03-1546) column is recommended for this application.
- The ZirChrom®-PBD column is nearly identical in chromatographic selectivity to the L-29 (Alumina-PBD) column.
- The use of a guard column is strongly recommended to extend column life and enhance column performance (Holder part # 850-00, Insert part # ZR03-G40).

Mobile Phase

- The use of highly basic buffers with ZirChrom®-PBD is recommended to enhance the performance of the assay.
- The recommended mobile phase is 29/71 Acetonitrile/14mM Potassium phosphate, pH 11, at a flow rate of 1.0 ml/min.

Detection Parameters

- Electrochemical detection may be used in aperometric mode with dual glassy carbon electrodes.
- Electrode 1 should be set at +.070V, electrode 2 at +0.82V.
- The background current should be set at 85 nA.

Column Loading Capacity

Using a 15 cm x 4.6 mm i.d. column, the following column capacities are indicated by a 10% reduction in k'.

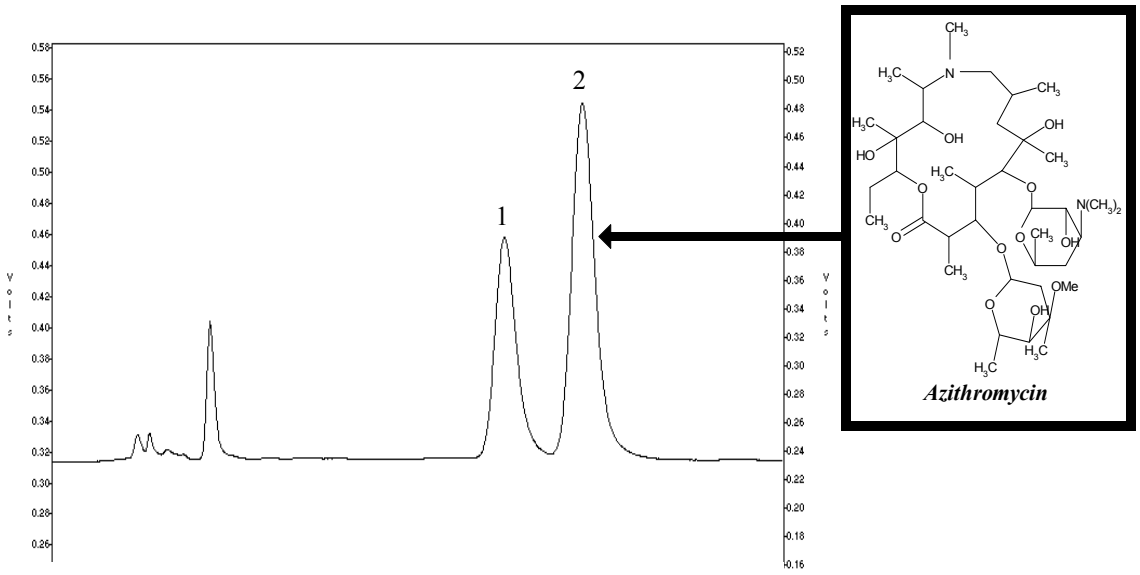
- Azithromycin — 2.0 mg/ml
- Azaerythromycin — 1.6 mg/ml

Analyte Detection Limits

Using the electrochemical detection scheme given above, the minimum detection limits are given below.

- Azithromycin — 4.7 ng/ml
- Azaerythromycin — 8.5 ng/ml

An analysis of Azithromycin and its analog Azaerythromycin is shown below.



LC Conditions: Column, ZirChrom®-PBD, 150 x 4.6 mm i.d.; Flow Rate, 1.0 ml/min.; Mobile Phase, 29/71 acetonitrile/14 mM potassium phosphate monobasic at pH 11; Column Temperature = ambient; Detector, amperometric electrochemical; Injection volume, 50 microliters; Sample concentration, 5 micrograms/ml. Solutes: 1 = azaerythromycin, 2 = azithromycin.

Analytical Performance

- The ZirChrom®-PBD column gives plate counts of better than 2000 plates/column, with asymmetry factors of 1.37 and 1.45 for azithromycin and azaerythromycin respectively.
- Calibration curves constructed using this analysis show excellent linearity over a concentration range of 0.0 to 10.0 micrograms/ml.
- This level of performance is similar to what is reported for L-29 (Alumina-PBD).
- Studies of column life at pH 11 for ZirChrom-PBD and Alumina-PBD have shown that ZirChrom®-PBD is stable for more than 10,000 column volumes of operation, while the Alumina-PBD fails after 2500 column volumes of use.
- ZirChrom columns exhibit a high degree of reprodubibility from column to column, making them an excellent choice for the application of the Azithromycin assay.

ZirChrom column are available worldwide. For on-line ordering and for a listing of distributors, please visit our website at http://www.zirchrom.com.

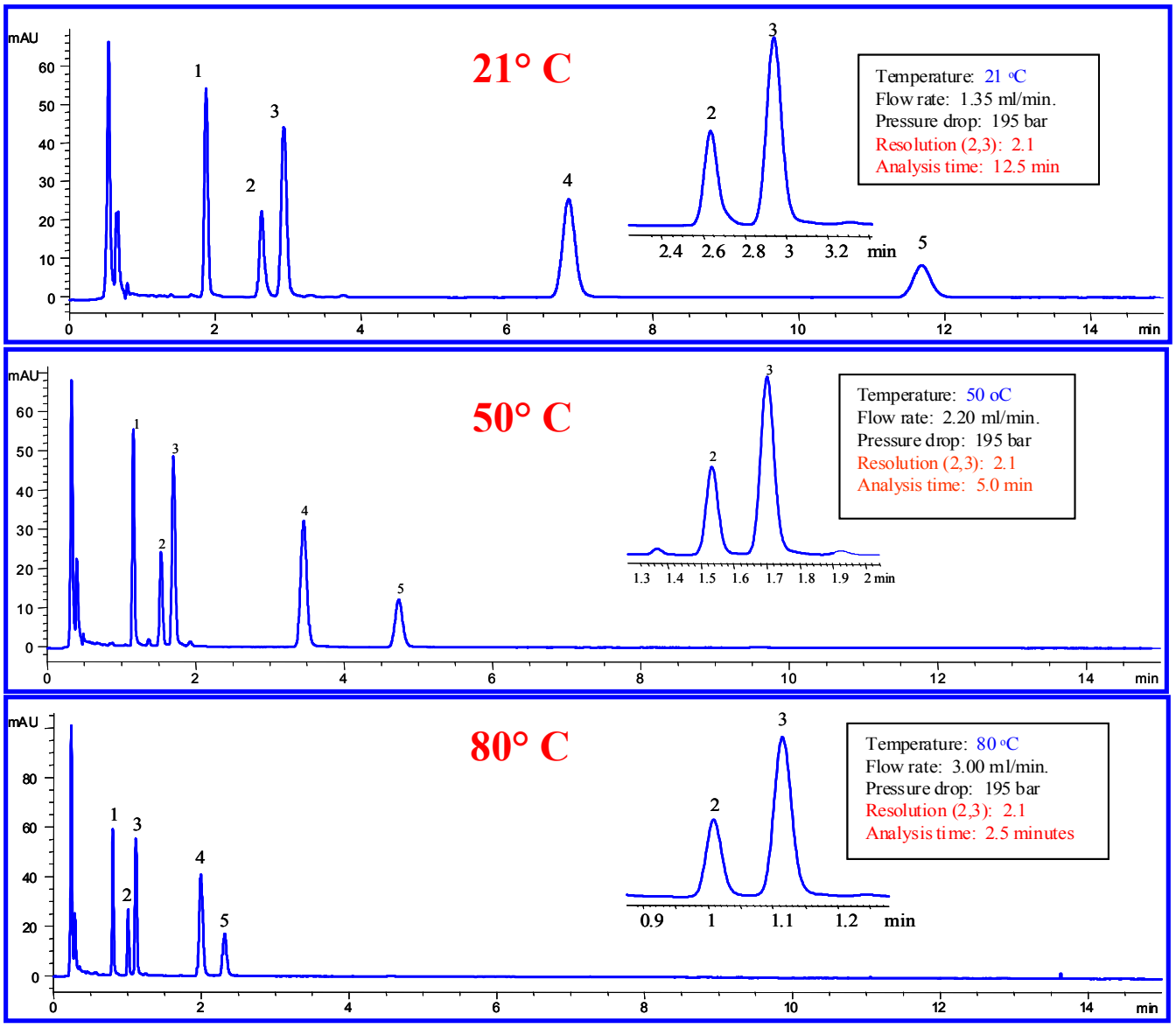
ZirChrom Separations, Inc.
617 Pierce Street
Anoka, MN 55303
Toll Free: (866) STABLE-1
Phone: (763) 421-5264
Fax: (763) 421-2319
Email: support@zirchrom.com
http://www.zirchrom.com

ZirChrom®

Technical Bulletin #259

... For Peak Performance

Ultra Fast High Temperature HPLC on ZirChrom®-PBD



Column: ZirChrom®-PBD 100mm x 4.6mm

Solutes: 1-Doxylamine, 2-Methapyrilene, 3-Chlorpheniramine, 4-Triprolidine, 5-Meclizine

Mobile Phase: 21° C - Isocratic 29/71 A/B

50 and 80° C - Isocratic 28/72 A/B

A: Acetonitrile

B: 50mM Tetramethylammonium hydroxide pH 12.2

Temperature: See individual runs

Injection volume: 0.2 µL

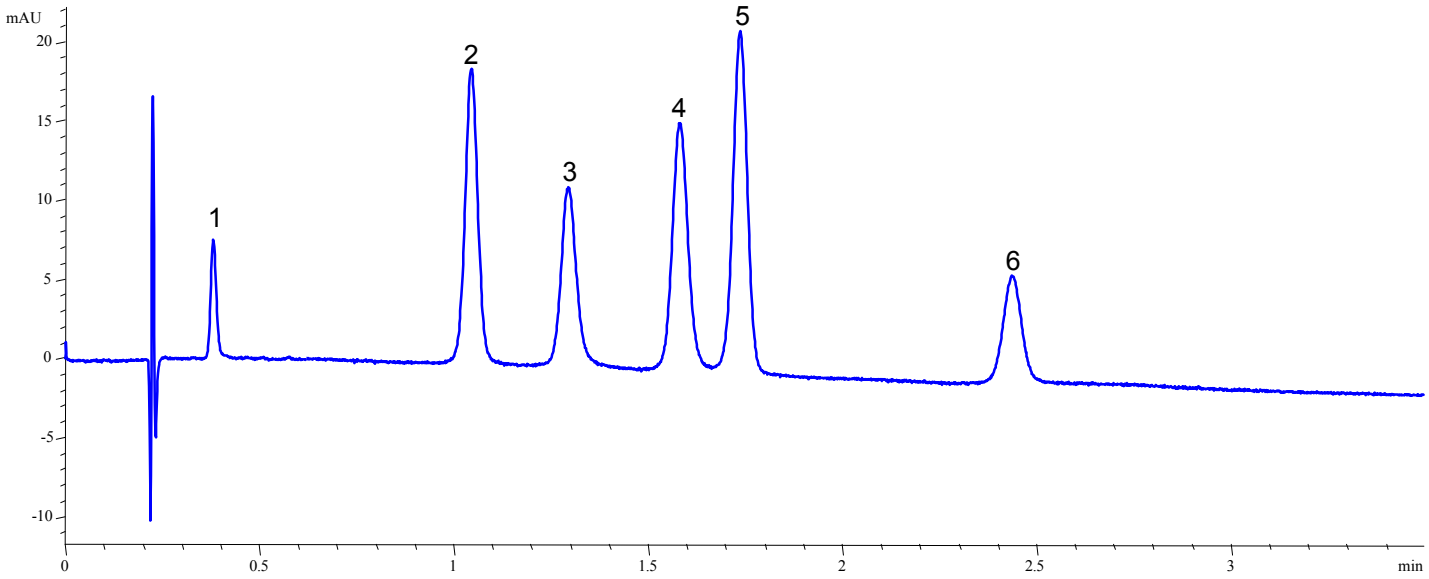
Detection: 254 nm

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

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

Flow rate:	2.5 mL/min.
Temperature:	40 ºC
Injection volume:	2 µL
Detection:	254 nm
Back Pressure:	175 bar

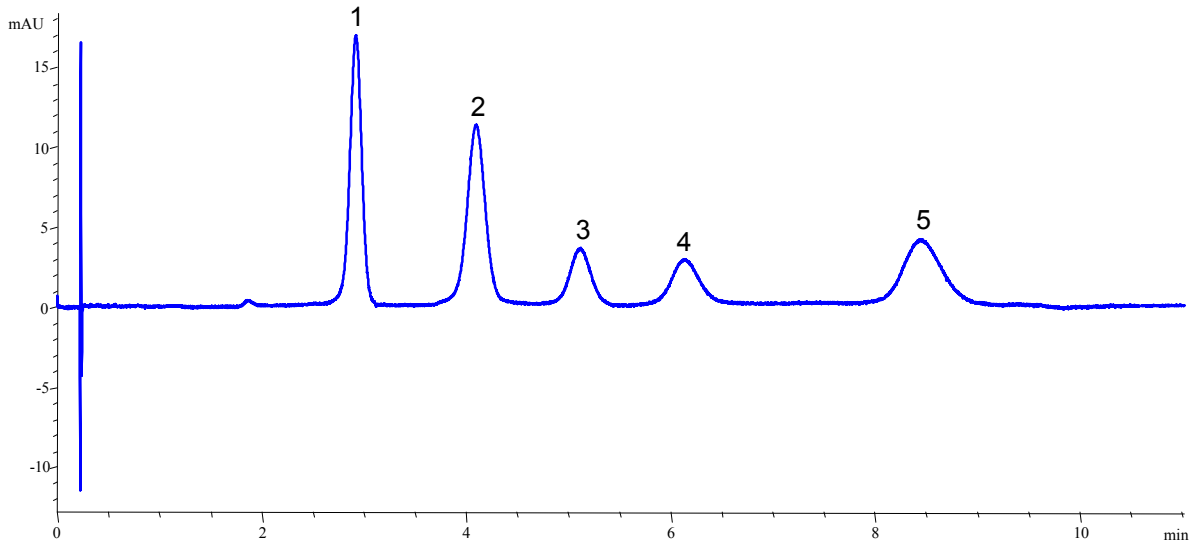
A: 20mM Acetic Acid pH to 5.0 using Ammonium Hydroxide
B: ACN

ZirChrom®

Technical
Bulletin #262

... For Peak Performance

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

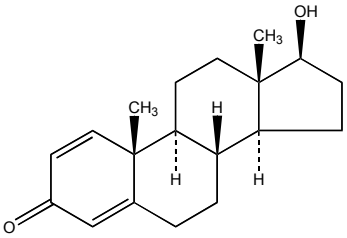
Flow rate:	2.5 mL/min.
Temperature:	40 ºC
Injection volume:	2 µL
Detection:	254 nm
Back Pressure:	175 bar

HPLC Separation of Anabolic Steroids on ZirChrom®-PBD

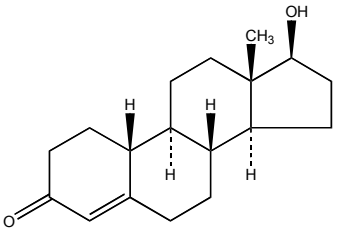
Dwight Stoll and Dr. Clayton V. McNeff
ZirChrom Separations, Inc.

Technical Bulletin #264

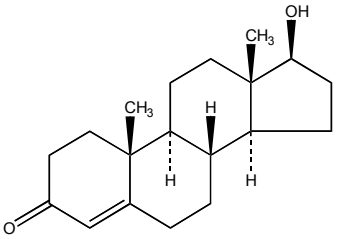
This note shows the separation of three closely related anabolic steroids, boldenone, nandrolone, and testosterone, using a ZirChrom®-PBD column. A typical analysis of these compounds involves derivatization and subsequent quantitation by GC-FID or GC-MS, however these methods tend to be labor intensive, and analytically unreliable. Baseline resolution of all three compounds was obtained on ZirChrom®-PBD at slightly elevated temperature in under 10 minutes using isocratic conditions.



Boldenone



Nandrolone



Testosterone

Figure 1: Structures of Anabolic Steroids

Introduction

The rapid and accurate detection of anabolic steroids is crucial in today's sporting world. Historically the structurally similarity of these compounds has made quantitative analysis by reversed-phase HPLC difficult at best. These steroids are very difficult to separate on silica ODS phases and provide nearly identical mass spectra. This method capitalizes on the unique temperature stability and surface chemistry of a zirconia-based stationary phase to achieve baseline resolution of these compounds in less than 10 minutes.

Experimental

A mixture of anabolic steroids was separated at 60 ºC using a ZirChrom®-PBD column (See Figure 2) using the following conditions.

Column:	150 mm x 4.6 mm i.d. ZirChrom®-PBD
Mobile Phase:	15/85 ACN/Water
Flow Rate:	1.5 ml/min
Injection Vol.:	5 µl
Pressure Drop:	160 bar
Temperature:	60 ºC with Metalox™ 200-C Column Heater
Detection:	UV at 215 nm

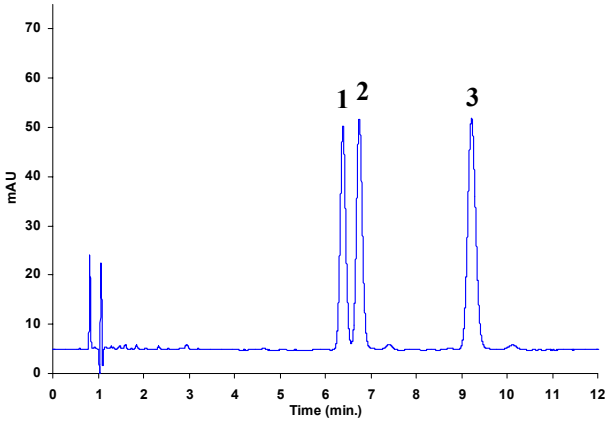


Figure 2: Separation of anabolic steroids on ZirChrom®-PBD. 1-Boldenone, 2-Nandrolone, 3-Testosterone

This separation allows for clear identification and quantification of these compounds without the use of expensive MS detection. The separation also requires no complex buffers and uses a minimum of organic modifier.

Acknowledgments

(1) Walter Hyde, Iowa State University

(2) Metalox Technologies, Inc.

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Faster Analysis and Higher Efficiency with Thermally-Stable HPLC Columns

Technical Bulletin #269

Dwight Stoll, Peter W. Carr
ZirChrom Separations, Inc.

Chromatographers have long known that modest increases in operating temperature can dramatically improve both the efficiency and speed of an HPLC separation. Until now, this potential has been unrealized because of the short lifetimes of high efficiency stationary phases at elevated temperature. This note shows that fast analysis with high efficiency is now easy to achieve using ultra-stable zirconia columns.

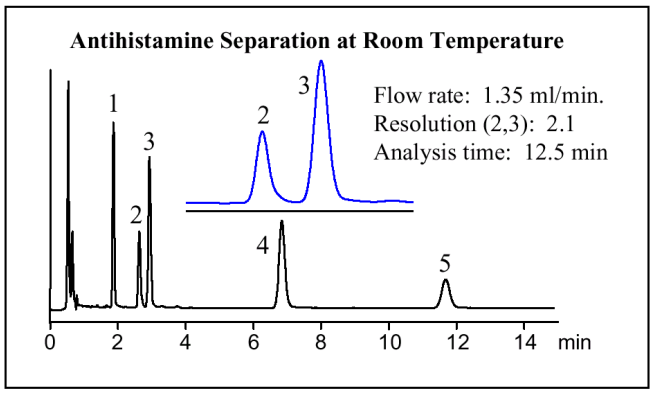

Figure 1: Separation of antihistamines at room temperature. 1=Doxylamine, 2=Methapyrilene, 3=Chlorpheniramine, 4=Triprolidine, 5=Meclizine

Introduction

In a recent article, David V. McCalley found large increases in the efficiency for basic compounds at elevated temperature[1]. McCalley suggested both that basic compounds should be analyzed at high temperature and that columns should be developed that are stable at high temperature.

In addition to improved efficiency, high temperature operation allows for dramatic improvements in analysis speed. Raising the temperature decreases mobile phase viscosity, allowing for increased eluent flow rate (and faster analysis) without excessive backpressure.

Experimental

A mixture of antihistamines was separated at room temperature using a ZirChrom®-PBD column. The separation conditions were as follows:

Column:	4.6 mm x 100 mm ZirChrom-PBD
Mobile Phase:	29/71 ACN / 50mM Tetramethylammonium hydroxide, pH 12.2
Injection Vol.:	0.5 ul
Pressure Drop:	195 bar
Detection:	UV at 254 nm

The initial separation is shown in Figure 1. Then, the temperature was increased to 50 °C, and the eluent flow rate also increased to maintain the same system backpressure. The separation (not shown) was more than twice as fast, with the resolution of the two closely eluting compounds maintained.

Finally, the temperature was increased to 80 °C, again increasing the eluent flow rate to maintain system backpressure. Now the separation is 5 times faster, maintaining the same resolution of the closely eluting peaks (see Figure 2).

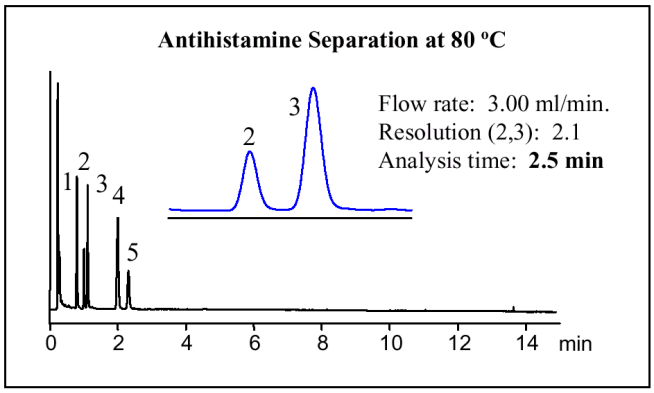

Figure 2: Separation of antihistamines at 80 °C. Analytes as in Figure 1.

Note that even temperature-sensitive compounds can benefit from modest increases in temperature, making faster analysis possible. ZirChrom's technical support group has extensive experience in this area, and would be happy to help you with your particular application.

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

References

(1) David V. McCalley, J. Chrom. A 902, 311-321 (2000).

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Technical Bulletin #272

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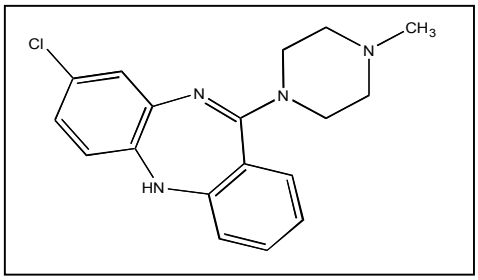
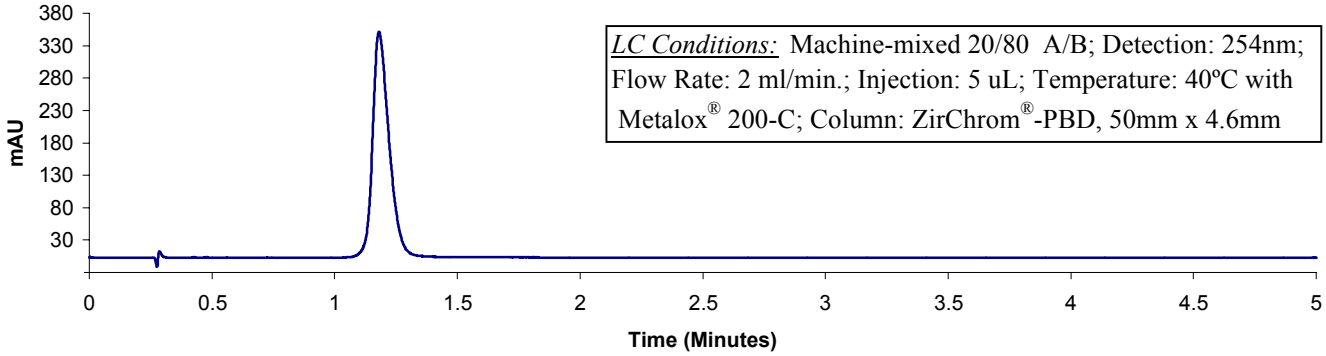
Chromatography of Clozapine on ZirChrom®-PBD

Clozapine and other compounds with similar structures present a challenge to chromatographers using silica phases. On ZirChrom®-PBD *symmetrical peaks can be obtained in a short run time using isocratic or gradient methodology.*

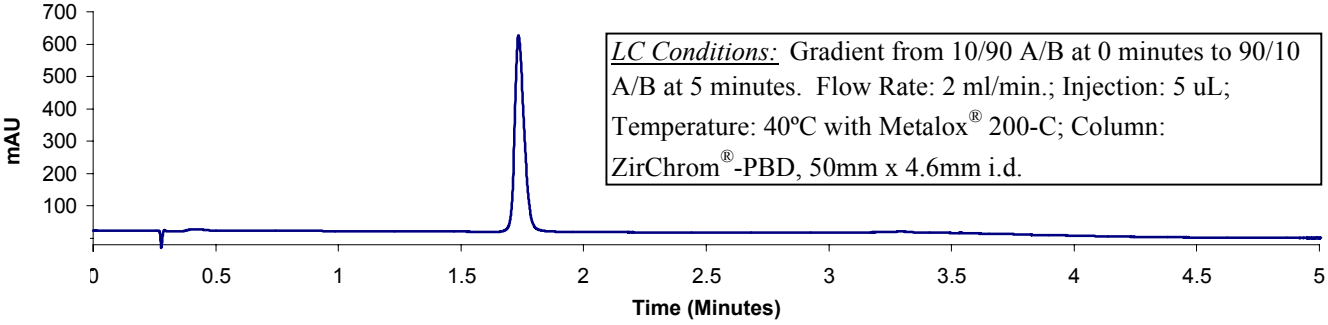


Isocratic



LC Conditions: Machine-mixed 20/80 A/B; Detection: 254nm; Flow Rate: 2 ml/min.; Injection: 5 uL; Temperature: 40ºC with Metalox® 200-C; Column: ZirChrom®-PBD, 50mm x 4.6mm

Gradient



LC Conditions: Gradient from 10/90 A/B at 0 minutes to 90/10 A/B at 5 minutes. Flow Rate: 2 ml/min.; Injection: 5 uL; Temperature: 40ºC with Metalox® 200-C; Column: ZirChrom®-PBD, 50mm x 4.6mm i.d.

Mobile Phase Preparation:

A: HPLC Grade Acetonitrile

B: 20mM Ammonium Acetate, 5mM Octylamine at pH 5.0 in Filtered, Degassed, Deionized Water

HPLC Analysis of Glyburide

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

Technical Bulletin #276

The fast analysis of glyburide was demonstrated using a LC/MS friendly buffer on a narrow-bore ZirChrom®-PBD column. The use of elevated temperature for the analysis reduces the eluent viscosity, thereby allowing higher flow rates and shorter analysis times, with only modest back pressure. Despite the fast analsis time, the retention factor of the analyte is preserved at the high flow rates, allowing the elution of potential matrix interferences before the analyte of interest.

Introduction

The structure of glyburide, an antihypoglycemic drug from the family of sulfonylureas, is shown in Figure 1. When taken in an oral dosage form, glyburide is effective in reducing high blood glucose levels.

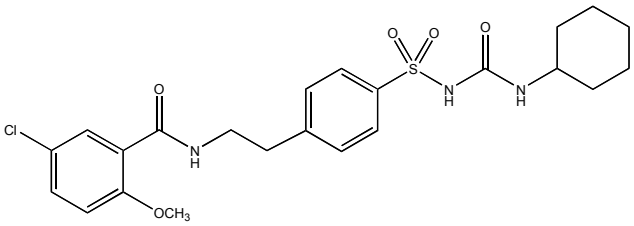


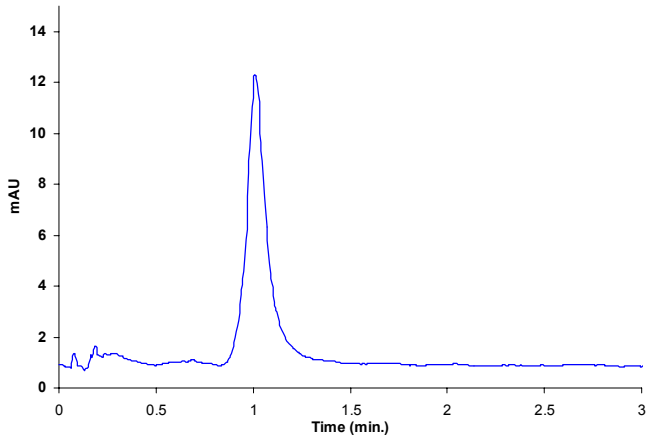
Figure 1. Structure of glyburide.

The routine analysis of glyburide by LC/MS requires a robust method that provides good peak shape, with adequate retention to allow for the separation of the analyte from matrix interferences which interefere with analyte ionization. The extraordinary chemical and physical stability of zirconia-based stationary phases allows temperature to be used as an operating variable in method development. This is particularly useful in this type of routine analysis, where the reduced eluent viscosities encountered at elevated temperatures allow use of higher flow rates and therefore shorter analysis times without appreciable loss in plate count which causes problems when analyses are speeded up by using shorter columns.

Experimental

A sample of glyburide (Sigma-Aldrich) was analyzed at slightly elevated temperature using a narrow-bore ZirChrom®-PBD column. The separation conditions were as follows:

Column:	2.1 mm x 50 mm ZirChrom®-PBD
Mobile Phase:	30/70 ACN/20mM Acetic acid, pH 3.3
Temperature:	70 °C
Injection Vol.:	1 µl
Pressure Drop:	126 bar
Detection:	UV at 240 nm
Flow Rate:	0.80 ml/min



Despite the relatively short retention time of glyburide, under these conditions the retention factor is 4.0 due to the use of high linear velocity. This retention factor provides sufficient separation space to allow for the elution of matrix components well before the glyburide peak, ensuring reliable detection by mass spectrometry.

It is important to note that many temperature-sensitive compounds can benefit from modest increases in temperature, making faster analysis possible. ZirChrom's technical support group has extensive experience in this area, and would be happy to help you with your particular application.

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

Acknowledgement

Jim Johnson, Acting Director of Bioanalytical Services, PRACS Institute, Fargo, ND

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HPLC Analysis of Rapamycin

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

Technical Bulletin #277

A fast gradient method was developed for the separation of rapamycin from an extracted human plasma matrix using a ZirChrom®-PBD column. The unique chemistry of this zirconia-based stationary phase allows for elution of the majority of the matrix interferences early in the gradient, while rapamycin elutes as a sharp, symmetrical peak in the middle of the gradient slope, free of interferences.

Introduction

The structure of rapamycin, also known as Sirolimus, is shown below in Figure 1. Rapamycin is one of a number of macrolide immunosuppresant drugs, and is commonly used in combination with other immunosuppresants such as cyclosporine (1). Previous reports have indicated that cis- and trans- isomer forms of rapamycin exist, often leading to split or double peaks in reversed-phase separations. This phenomenon has been observed by UV detection, and the identity of both peaks as rapamycin has been confirmed by LC/MS (1,2).

Previous reports of the analysis of rapamycin by UV detection have commented on overly long analysis times (45 minutes) mainly due to matrix interences (3). Several reports of detection by mass spectrometry have indicated analysis times of 10-15 minutes for plasma samples (1,4).

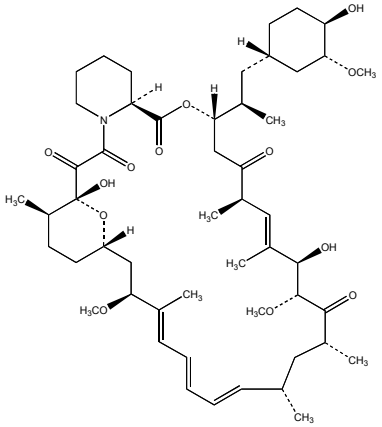

Figure 1. Structure of rapamycin

Experimental

The chromatographic conditions for the analysis of rapamycin were as follows:

Column:	50 mm x 4.6 mm id. ZirChrom®-PBD
Mobile phase:	Gradient elution from 5-95% B over 5 minutes A: 20mM Ammonium phosphate, pH 5.0 B: Acetonitrile
Flow Rate:	2.0 ml/min.
Temperature:	75 °C
Detection:	278 nm
Inj. Volume:	5 µl

Chromatograms obtained by injecting a rapamycin standard (blue trace), and an extracted plasma sample (red trace) are shown in Figure 2. We note that the column temperature in this separation is 75 °C, which is important to obtain good peak shape for this compound.

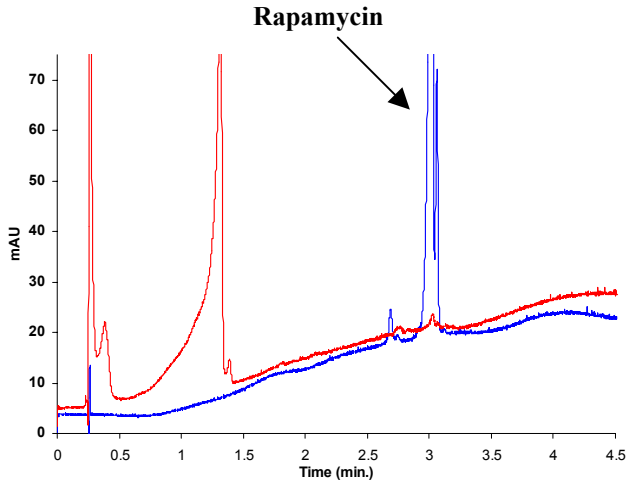

Figure 2: Analysis of rapamycin standard (blue trace), and rapamycin in extracted plasma sample (red trace).

In the chromatogram for the rapamycin standard, a small shoulder is often observed on the tail of the peak and is most likely an isomer of the main peak.

In the red trace it is clear that all of the matrix components are eluting very early in the gradient, eliminating any potential interferences with quantitation of the rapamycin.

ZirChrom columns combine the high efficiency usually associated with silica columns with unsurpassed chemical and thermal stability, resulting in this extraordinarily fast and highly selective analysis.

References

(1) G. Kirchner, et al. J. Chrom. B: Biomed. Appl., 721, 1999, 285-294.
(2) I. Segarra, et al. J. Chrom. B: Biomed. Appl., 720, 1998, 179-187.
(3) K. Napoli, et al. J. Chrom. B: Biomed. Appl., 654, 1994, 111-120.
(4) P. Taylor, et al. J. Chrom. B: Biomed. Appl., 718, 1998, 251-257.

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Fast Separation of Mono and Di-esters from BPA(EO)30DMA Methacrylated Polyol

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

Technical Bulletin # 292

This technical bulletin details the separation of mono and di-esters from BPA(EO)30DMA methacrylated polyol. The current method for Surface Specialties UCB's analysis of the mono- to di-ester ratio in a final polymer product, specifies an HPLC method which is 30 minutes in length. However, their current method required two injections per process sample, thereby bringing the total analysis cycle time to approximately 60 minutes. Here we report a new method using ZirChrom®-PBD at a column temperature of 80ºC with a total analysis time of only 10 minutes.

Introduction

Surface Specialties UCB is a company located in Smyrna, Georgia that manufacturers specialty chemicals, coating resins, additives, adhesives, and technical resins. Surface Specialties UCB approached ZirChrom method developers with the challenge of shortening the run time of an analysis of mono- to di- ester ratio in a final polymer product. Their current method for the analysis of the mono- to di-ester ratio in a final polymer product specifies an HPLC method less than 30 minutes in length. In practice, however, two injections are required for each process sample bringing the total analysis time to approximately 60 minutes. Since the HPLC analysis is used for online monitoring of the reaction, a 60 minute analysis time meant it was too late to stop a reaction that had gone too far. ZirChrom Solution: ***Use a Zirchrom®-PBD column and elevated temperature to speed up the analysis!***

Experimental

A mixture of mono and di-esters from BPA(EO)30DMA methacrylated polyol was separated at 80 ºC using a ZirChrom®-PBD column. The separation conditions were as follows:

Column: ZirChrom®-PBD, 150 mm x 4.6 mm i.d. (Part Number: ZR03-1546)
Mobile Phase: A: water
B: acetonitrile

Time	%A	%B
0	80	20
5	20	80

Temperature: 80 ºC with Metalox® 200-C column heater
Flow Rate: 1.5 ml/min.
Injection Vol.: 5 µl of 1 mg/ml sample
Pressure Drop: 155 bar
Detection: UV at 240 nm

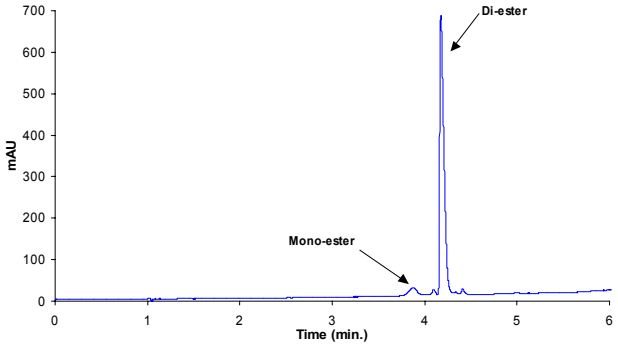

Figure 1: *Separation of Mono and Di-ester at 80 °C using ZirChrom®-PBD and the Metalox® 200-C.*

Customer Allyson Norman, Analytical Scientist with Surface Specialties UCB had this to say about the ZirChrom method; "ZirChrom method developers reduced our analysis turn around time from 1 hour to 10 minutes!"

The ZirChrom value assessment program: (available on the ZirChrom website: http://www.zirchrom.com/documents/value.xls) predicts reducing the total analysis time from 60 minutes (30 minutes/injection) to 10 minutes (5 minute/injection) will save UCB roughly $20.00/sample in total analysis costs. This translates to roughly $158,600/year in savings (savings based on possible cycles/instrument/year using the ZirChrom column). This savings calculation takes into account the cost of purchasing a Metalox® 200-C high temperature column heater and a ZirChrom®-PBD column. Although the cost savings alone are impressive, having the analysis completed before the reaction has gone too far is *priceless*.

This method can be tailored to your specific application needs. ZirChrom technical support can help to optimize and transfer this technology to your site. Please contact ZirChrom technical support at 1-866-STABLE-1 or support@zirchrom.com for details. For more information on the Metalox® 200-C high temperature column heater visit www.metalox.com.

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

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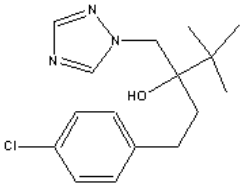
Fast Separation of Wood Preservatives on ZirChrom®-PBD

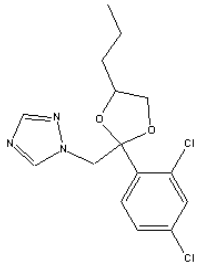
Kelly S. Johnson and Dr. Clayton McNeff
ZirChrom Separations, Inc.

Technical Bulletin # 294

Overlapping phenolic compounds complicate the analysis of the preservatives tebuconazole and propiconazole in wood extract samples. A mobile phase chosen to take advantage of the mixed-mode separation capability of ZirChrom®-PBD speeds and simplifies this separation. Tebuconazole and propiconazole standards were separated on a ZirChrom®-PBD column at 40ºC using a mobile phase optimized to separate the two preservatives while preventing the interference of phenolic compounds also present in real world samples.



Tebuconazole



Propiconazole

Figure 1. Structures of Tebuconazole and Propiconazole

Introduction

Tebuconazole and propiconazole are water-based wood preservatives that prevent decay from fungi in millwork, shingles and shakes, siding, plywood, structural lumber and timbers, and composites that are used in above ground applications. A ZirChrom customer contacted technical support for assistance with the quantification of these compounds from a wood extract sample. The customer reported problems using a silica-gel based column due to interfering phenolic compounds. A new separation was developed to take advantage of the inherent surface chemistry differences of a zirconia-based column when tackling interfering peaks or closely related compounds. Phenolic compounds are known to be Lewis bases; thus a phosphate containing mobile phase is used to prevent the adsorption to Lewis acid sites on the surface of zirconia. In this case, the effect of using phosphate buffer on zirconia is twofold: 1) phosphate will adsorb to bare zirconia sites and 2) phosphate will create a negatively charged surface that will exclude the ionized phenols from the Lewis base negatively charged particles. This results in the phenolic compounds being eluted slightly before or at the deadtime (unretained), while the analytes of interest are retained and resolved. Finally, additional salt (100mM NaCl) was added to the mobile phase to reduce retention due to the ion-exchange mode of retention of the positively charged analytes. The resulting separation achieved baseline resolution in less than four minutes while allowing quantification of these analytes without the interference of other matrix compounds.

Experimental

Tebuconazole and propiconazole were separated at 40 ºC using a ZirChrom®-PBD column. The separation conditions were as follows:

Column:	ZirChrom®-PBD, 150 mm x 4.6 mm i.d. (Part Number: ZR03-1546)
Mobile Phase:	40/60 acetonitrile/20mM ammonium acetate, 20mM ammonium phosphate, 100mM NaCl pH 5.00
Temperature:	40 ºC with Metalox™ 200-C column heater
Flow Rate:	1 ml/min.
Injection Vol.:	5 µl
Pressure Drop:	195 bar
Detection:	UV at 223 nm

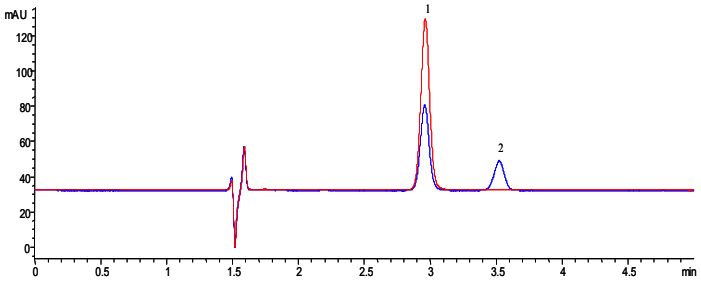


Figure 2. Separation of Wood Preservatives: BLUE TRACE, 1 – Tebuconazole, 2 – Propiconazole; RED TRACE, 1 – Tebuconazole

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.

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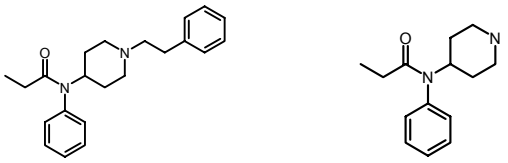
Simultaneous Extraction and Quantitation of Fentanyl and Norfentanyl from Primate Plasma with LC/MS Detection

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

Technical Bulletin # 300

The quantitation of fentanyl and its desalkyl metabolite, norfentanyl, in blood plasma using LC/MS detection has not been previously described. This application note reports the successful detection and quantitation of these basic drugs using a Zirchrom®-PBD column. Mass spectroscopy detection was performed using ESI in the positive mode. The LOQ for fentanyl was 25 pg/ml and norfentanyl was 50 pg/ml.



Fentanyl Norfentanyl

Figure 1. Structures of Fentanyl and Norfentanyl

Introduction

Transmucosal fentanyl is an analgesic agent used in the control of cancer pain in humans and as a presurgical sedative for children [1,2]. This method was developed by the Zoological Pharmacology Laboratory, College of Veterinary Medicine, Kansas State University (Manhattan, Kansas, USA) to support a pharmacokinetic/pharmacodynamic study of transmucosal fentanyl as a preanesthetic in chimpanzees, orangutans, and gorillas [3]. Along with obtaining data on fentanyl plasma concentrations, it was also desirable to have information on the metabolism of fentanyl in these three species of primates.

There are currently no published extraction and detection procedures that quantitate both fentanyl and norfentanyl from plasma using LC/MS. Fentanyl in plasma has been quantitated using LC [4] and radioimmunoassay [2]. Furthermore, the lowest published level of detection for fentanyl in plasma was 100 pg/ml. The assay reported here allowed quantitation to 25 pg/ml for fentanyl and 50 pg/ml for norfentanyl. The liquid-liquid extraction used toluene as the organic phase [5].

Experimental

A mixture of fentanyl and norfentanyl was separated at room temperature using a ZirChrom®-PBD column and an LCQ_{DUO} LC/MS system manufactured by ThermoFinnigan (San Jose, CA) using an ESI source with positive ionization. The separation conditions were as follows:

Column:	ZirChrom®-PBD, 50 mm x 2.1 mm i.d., 3 micron (Part Number: ZR03-0521)
Mobile Phase:	45/55 (v/v) acetonitrile/10 mM ammonium acetate, 0.1 mM citrate (pH 4.4)
Temperature:	Uncontrolled
Flow Rate:	0.3 ml/min.
Injection:	50 µl
Detection:	LC/MS/MS

This method results in a sensitive and accurate assay that allows for the quantitation of both fentanyl and norfentanyl from primate plasma. The liquid-liquid extraction combined with the sensitivity of MS detection has allowed lower quantitation concentrations of both compounds than previously reported [5].

Table 1: Chromatographic Results for Fentanyl and Norfentanyl

Compound	Retention Time	k'
Fentanyl	2.24	2.11
Norfentanyl	4.86	5.75

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

D.E. Koch, R. Isaza, J.W. Carpenter, and R.P. Hunter, Zoological Pharmacology Laboratory, College of Veterinary Medicine, Kansas State University (Manhattan, Kansas, USA)

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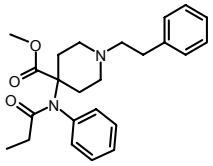
Extraction and Quantitation of Carfentanil and Naltrexone in Mammalian Plasma with LC/MS Detection

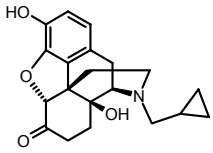
Clayton McNeff, Ph.D. and Steven Rupp
ZirChrom Separations, Inc.

Technical Bulletin # 301

The quantitation of carfentanil and naltrexone at pharmacologically relevant plasma concentrations has not been previously described. This application note reports the sensitive and accurate detection and quantitation of these basic drugs by LC/MS using a ZirChrom®-PBD column. The ability to detect and quantitate carfentanil and naltrexone with a single extraction dramatically decreases the time and money needed to perform sample analysis, especially since both drugs are used concurrently in zoological medicine.



Carfentanil



Naltrexone

Figure 1. Structures of Carfentanil and Naltrexone

Introduction

Carfentanil (CARF) is the most potent opioid agonist currently in use. It is 20× more potent than fentanyl [1], and is approved by the United States Food and Drug Administration for immobilization of free-ranging or confined members of the family Cervidae (i.e. white-tailed deer, elk, & moose). Since its development in 1975, CARF has become the drug of choice for immobilization of a wide variety of non-domestic mammals [1,2], because it allows for rapid and reliable induction of anesthesia with small volumes of CARF in a diverse range of species [3]. Carfentanil is a synthetic derivative of fentanyl (refer to Technical Bulletin # 300). In most situations, CARF anesthesia is reversed using the antagonist naltrexone (NLT) [1].

This analytical method was developed by the Zoological Pharmacology Laboratory, College of Veterinary Medicine, Kansas State University (Manhattan, Kansas, USA) to quantitate the plasma concentrations of carfentanil and naltrexone using a single toluene-based extraction method [4]. The sensitivity of this method is two orders of magnitude lower than previously reported methods [5]. It will assist with providing information on the pharmacokinetics of these compounds.

Experimental

A mixture of carfentanil and naltrexone was separated at room temperature using a ZirChrom®-PBD column and an LCQ_{DUO} LC/MS system manufactured by ThermoFinnigan (San Jose, CA) using an ESI source with positive ionization. The separation conditions were as follows:

Column:	ZirChrom®-PBD, 50 mm x 2.1 mm i.d., 3 micron (Part Number: ZR03-0521)
Mobile Phase:	30/70 (v/v) acetonitrile/10 mM ammonium acetate, 0.1 mM citrate (pH 4.4)
Temperature:	Uncontrolled
Flow Rate:	0.3 ml/min.
Injection:	50 µl
Detection:	LC/MS/MS

The use of LC/MS allows for the determination of plasma levels of carfentanil prior to and following reversal of anesthesia with greater sensitivity and confidence. The limit of quantitation was 8.5 pg/mL for carfentanil and 0.21 ng/mL for naltrexone [4].

Table 1: Chromatographic Results for Carfentanil and Naltrexone

Compound	Retention Time	k'
Naltrexone	~ 1.7	~ 1.5
Carfentanil	~ 2.7	~ 3.0

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.

Acknowledgements

R.P. Hunter, D.E. Koch, A. Mutlow, and R. Isaza, Zoological Pharmacology Laboratory, College of Veterinary Medicine, Kansas State University (Manhattan, Kansas, USA)

References

[1] M.E. Fowler, in Restraint and Handling of Wild and Domestic Animals, Iowa State Univ. Press, Ames, Iowa, 1995, Ch. 4, p. 36.

[2] J.L. Allen, J.E. Janssen, J.E. Oosterhuis, & T.H. Stanley, Proceedings of the Annual Meeting of the American Association of Zoo Veterinarians, 1991, p. 343.

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A Simple and Sensitive HPLC Method for the Detection and Quantitation Of STI-571 And Its Main Metabolite N-Desmethyl-STI

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

Technical Bulletin # 302

An isocratic online enrichment HPLC-assay was developed allowing for the simple and fast separation and quantitation of STI-571 and its main metabolite N-Desmethyl-STI in plasma, urine, cerebrospinal fluid, culture media and cell preparations in various concentrations using UV-detection at 260 nm. The analytical procedure consists of an online concentration of STI-571 and N-Desmethyl-STI in the HPLC system followed by the elution on a ZirChrom®- PBD analytical column.

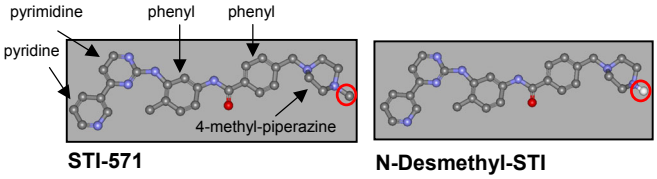

Figure 1. Structures of STI-571 and N-Desmethyl-STI

Introduction

STI-571 (Imatinib mesylate, Glivec™), a 4-[(4-methyl-1-piperazinyl)methyl]-N-[4- [1–3] methyl - 3 - [[4 - (3 - pyridinyl) - 2 - pyrimidinyl]amino]- phenyl] benzamide methane-sulfonate derivative, acts as an inhibitor of the abl tyrosine kinase, platelet derived growth factor receptor (PDGFR), stem cell factor receptor (c-kit, steel factor receptor, CD117) and ARG tyrosine kinases. The specific blockade of the bcr-abl oncoprotein has been associated with significant antileukemic activity in patients with chronic-myeloid-leukemia (CML) and Philadelphia-positive-acute-lymphatic-leukemia (Ph+ALL).

This analytical method was developed by Universitätsklinikum Carl Gustav Carus an der Technischen Universität (Dresden, Germany) to perform pharmacokinetic measurements of STI-571 and N-Desmethyl-STI in patient samples (plasma, urine, cerebrospinal fluid) and for kinetic measurements of intracellular STI-571 and N-Desmethyl-STI following in-vitro incubation [1]. This method utilizes UV detection but may also be adapted to electrochemical detection to enable lower detection limits.

Experimental

A mixture of STI-571 and N-Desmethyl-STI was separated at room temperature using a ZirChrom®-PBD guard column, a ZirChrom®-PBD analytical column and UV detection. This analytical method includes an online-enrichment system incorporating another ZirChrom®-PBD guard column and an electric motor driven switching valve (refer to [1] for schematic valve switching graph). The separation conditions were as follows:

Enrichment

Guard Column:	ZirChrom®-PBD, 10 mm x 4.6 mm i.d. Guard Insert (Part Number: ZR03-G40; set of 3); Guard Insert Holder (Part Number 850-00)
Mobile Phase:	45/35/20 (v/v) 0.1 M dibasic potassium phosphate/water/methanol
Temperature:	Uncontrolled

Analytical

Guard Column:	ZirChrom®-PBD, 10 mm x 4.6 mm i.d. Guard Insert (Part Number: ZR03-G40; set of 3); Guard Insert Holder (Part Number 850-00)
Column:	ZirChrom®-PBD, 50 mm x 4.6 mm i.d. (Part Number ZR03-0546)
Mobile Phase:	60/40 (v/v) 10 mM dibasic potassium phosphate, 90 mM monobasic potassium phosphate/methanol
Temperature:	Uncontrolled
Flow Rate:	0.4 ml/min.
Injection:	50 µl
Detection:	UV at 260 nm

Time of analysis is 40 minutes including the enrichment time of 5 minutes. The UV detection limit is 10 ng/ml in plasma, CSF (cerebrospinal fluid), culture medium (RPMI) and 25 ng/ml in urine for both STI-571 and N-Desmethyl-STI.

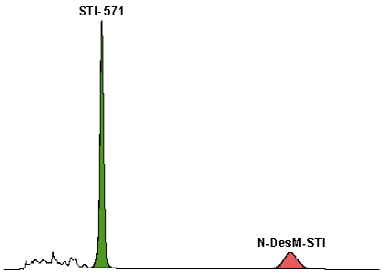

Figure 2: Chromatogram of patient plasma under STI-571 treatment with 5308 ng/ml STI-571 (RT ≈ 10.0 min.) and 988 ng/ml N-Desmethyl-STI (RT ≈ 30.0 min.).

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.

Acknowledgements

Universitätsklinikum Carl Gustav Carus an der Technischen Universität (Dresden, Germany)

References

[1] E. Schleyer, S. Pursche, C.H. Köhne, U. Schuler, U. Renner, H. Gschaidmeier, J. Freiberg-Richter, T. Leopold, A. Jenke, M. Bonin, T. Bergemann, P. le Coutre, M. Gruner, M. Bornhäuser, O.G. Ottmann, G. Ehninger, J. Chromatogr. B, 799, (2004) 23-36.

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Analysis of Azithromycin using ZirChrom®-PBD

Bingwen Yan, Ph.D. and Kelly Johnson
ZirChrom Separations, Inc.

Technical Bulletin # 311

The ZirChrom®-PBD column has been designated by the USP as L49 and can be used for the analysis of azithromycin. The retention time of azithromycin is very sensitive to small changes in mobile phase composition and in the surface chemistry of the ZirChrom®-PBD column. This application note lists helpful method suggestions to ensure good peak shape and consistent results.

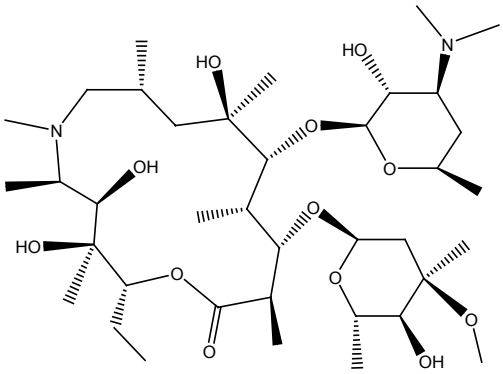

Figure 1: Structure of azithromycin.

Introduction

Azithromycin is a macrolide antibiotic that interferes with the growth of bacterial cells. Macrolides have activity against many gram-positive bacteria (excluding enterococci and methicillin-resistant *Staphylococcus aureus*), and have variable activity against respiratory gram-negative pathogens, *Mycobacterium avium* infections, gonorrhea, and *Chlamydia* infections (2). Azithromycin is used to treat bacterial infections in many different parts of the body but most often used to treat respiratory infections in children and adults.

The high pH necessary for the analysis of azithromycin prohibits the use of traditional silica based substrates and necessitates the use of the pH stable zirconia-based ZirChrom®-PBD. Azithromycin and its impurities lack good chromaphores and thus any analytical study of purity must be made using an electrochemical detector. For the purpose of this study we have injected a very concentrated sample and detected in the UV to avoid any issues inherent with that method of detection.

Experimental

USP standard solution of azithromycin was injected at 30ºC using a ZirChrom®-PBD column (see figure 2). The separation conditions were as follows:

Column: ZirChrom®-PBD, 150 mm x 4.6 mm i.d. (Part Number: ZR03-1546)
Mobile Phase: 5.8 g monobasic potassium phosphate in 2130 mL of water, added to 870 mL of acetonitrile adjusted to pH 11.0 with potassium hydroxide
Temperature: 30 ºC with Metalox™ 200-C column heater
Flow Rate: 1 ml/min.
Injection Vol.: 5 µl, 1 mg/mL
Pressure Drop: 195 bar
Detection: UV at 215 nm

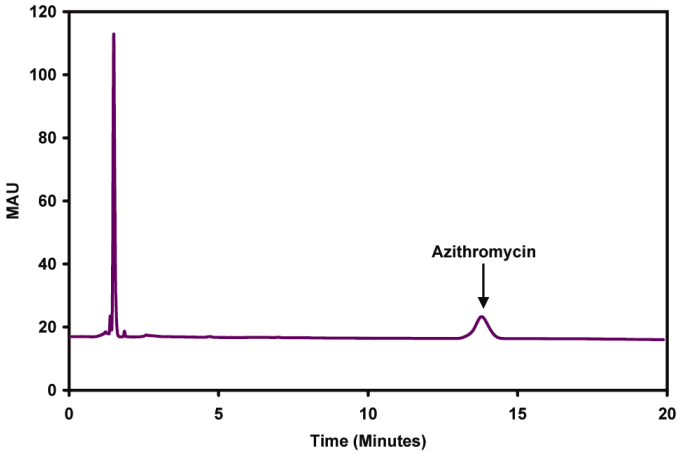

Figure 2: USP standard azithromycin.

Several method conditions were found to affect both the retention time and peak shape of the analyte:

1. Over-degassing of the mobile phase: The retention time of azithromycin is sensitive to both the concentration of phosphate and the percentage of organic, thus it is critical that these remain constant. We recommend degassing the aqueous and organic portions of the mobile phase separately then combining to adjust pH. This prevents loss of organic and changes in phosphate concentration. We also recommend a short flush of the column with pure water before equilibrating the column in the mobile phase.
2. Mobile phase left in column without flow: This mobile phase CANNOT be left in the column for any length of time without flow. The phosphate in the mobile phase will precipitate out into the column without flow and when flow is resumed it will dissolve back into the mobile phase leaving voids and greatly deteriorated peak shapes.
3. Uncontrolled column temperature: Placing the column in a thermostatted environment prevents retention fluctuations due to changes in ambient temperature and drafts.
4. Changes in pH: Small changes in pH can cause significant changes in retention time. It is very important to adjust up to but not over pH 11.0 $\pm$ 0.1 as indicated in the USP method. The pH will jump sharply for a period during adjustment and the potassium hydroxide must be added drop wise to prevent over correction.
5. Injection before complete equilibration: The column takes approximately 4-5 hours at 1 ml/min to fully equilibrate in the mobile phase buffer. Equilibration was defined as the stabilized retention time of azithromycin (see Figure 3). As this equilibration time is often equal to or less than the equilibration time of the electrochemical detector, it does not usually pose a significant issue.
6. Use of other mobile phase components and conditions: The use of different mobile phase components (such as ammonium phosphate or dibasic potassium phosphate) and pH conditions will change both the column surface chemistry and its interactions with the analyte. These changes can greatly effect equilibration and retention times.

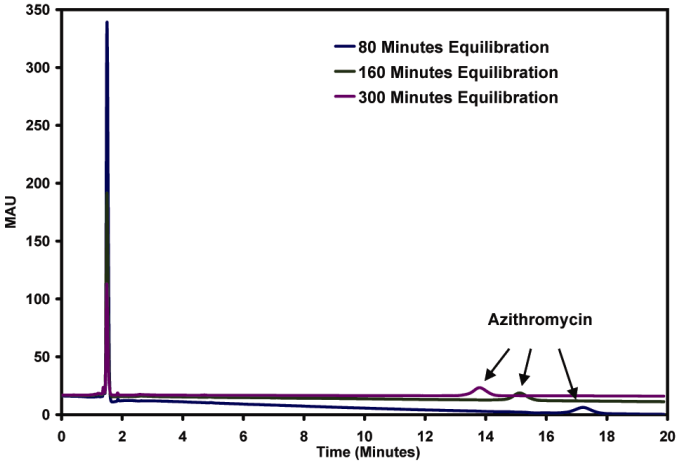

Figure 3: Affect of column exposure to USP mobile phase (equilibration) on the retention time of azithromycin.

Please contact ZirChrom technical support at 1-866-STABLE-1 or support@zirchrom.com with any additional questions or concerns.

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

References

(1) USP, USP Monograph: Azithromycin (2005).

(2) http://www.pfizer.com/download/uspi_zithromax.pdf

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Fast Separation of Ten Triazine Herbicides

Adam Schellinger and Dr. Peter Carr
University of Minnesota

Technical Bulletin # 318

This application note shows the separation of ten related triazine herbicides using a ZirChrom®-PBD column. Baseline resolution of all ten compounds was obtained at elevated column temperature in under 3 minutes using LC/MS compatible gradient elution.

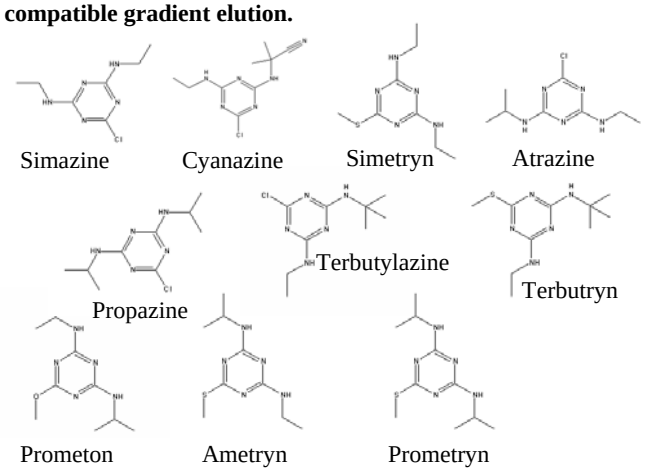

Figure 1: Structures of triazine herbicides

Introduction

Historically, the preferred analysis method for triazines, like many herbicides, has been gas chromatography. Although HPLC techniques are non destructive, allow for large volume injections (on column enrichment), and allow the use of a wide range of detectors, their use in this application has been hindered by long run times necessary to achieve the desired resolution. Previous method development efforts incorporating zirconia-based HPLC columns to separate these compounds achieved baseline resolution with a two-column T^3C approach, but run times still exceeded 10 minutes (1). Here we present a new HPLC method that exploits the unique selectivity and thermal stability of the ZirChrom®-PBD column to separate ten triazine herbicides in less than three minutes, four times faster than the previous method, with a LC-MS compatible mobile phase.

Experimental

A mixture of ten triazine herbicides was separated at 95 °C using a ZirChrom®-PBD column. The separation conditions were as follows:

Column: ZirChrom®-PBD, 75 mm x 3.0 mm i.d.
(Part Number: ZR03-0730)
Mobile Phase: Gradient Elution:
A: Water, B: Acetonitrile

Time (min)	% A	%B
0.00	100	0
4.75	60	40

Temperature: 95 ºC with Metalox™ 200-C column heater
Pressure Drop: 360 bar
Detection: UV at 254 nm

We report here a method that capitalizes on the unique temperature stability and surface chemistry of a zirconia-based stationary phase to achieve baseline resolution of these compounds in less than 3 minutes.

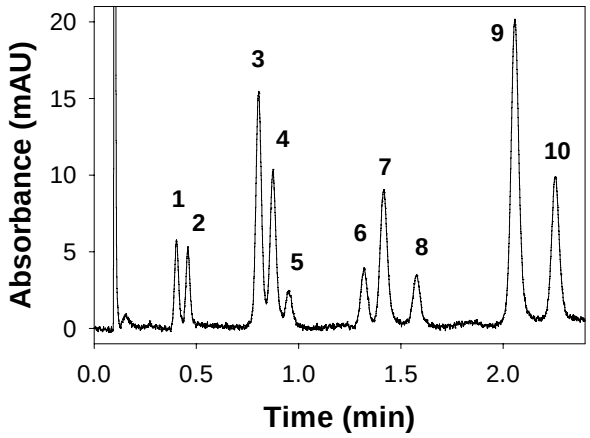

Figure 2: Separation of 1) Simazine; 2) Cyanazine; 3) Simetryn; 4) Atrazine; 5) Prometon; 6) Propazine; 7) Ametryn; 8) Terbutylazine; 9) Prometryn; 10) Terbutryn on a ZirChrom-PBD in under 3 minutes at 95 ºC

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) Mao, Y.; Carr, P.W.; Anal. Chem. 2000, 72(13), 2788-2796.

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Performance Analysis of ZirChrom®-PBD Sub-2µm Particles

Bingwen Yan, Ph.D. and Richard Henry, Ph.D. (consultant)
ZirChrom Separations, Inc.

Technical Bulletin # 327

As the capabilities of HPLC equipment increase so does the popularity of sub-2µm particles. In this application note we introduce our new line of sub-2µm particles and analyze the performance of our ZirChrom®-PBD phase.

Introduction

Since about 1970, there has been a steady effort to develop smaller particle particles that improve HPLC column efficiency. The most recent results with sub-2µm particles have been given the name Ultra-HPLC (UHPLC). The unparalleled thermal, chemical and mechanical stability of zirconia-based phases make them particularly well suited to this application.

Experimental

A mixture of five alkylbenzenes were separated at 30 ºC using a ZirChrom®-PBD column. The separation conditions were as follows:

Column:	ZirChrom®-PBD, 50 mm x 4.6 mm i.d. (Part Number: ZR03-0546-1.9)
Mobile Phase:	as noted on figure
Temperature:	30 ºC
Instrumentation:	HP1100 Thermostated Chemstation, Micro Cell, 0.007'' i.d. tubing
Detection:	UV at 254 nm

Results

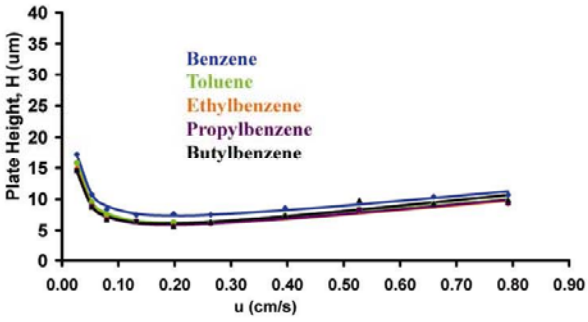

Figure 1. Plate height for 3µm ZirChrom®-PBD based on van Deemter equation vs. linear velocity for alkylbenzenes; Mobile Phase: 55/545 ACN/water

Figures 1-3 show comparison flow studies for 3µm and sub-2µm ZirChrom®-PBD. As theoretically expected, the sub-2µm particles are more efficient than the 3µm particles, especially at higher flow rates. The resistance to mass transfer is reduced with the small particles thus enabling a faster analysis (i.e. faster flow rate) without significant loss of efficiency.

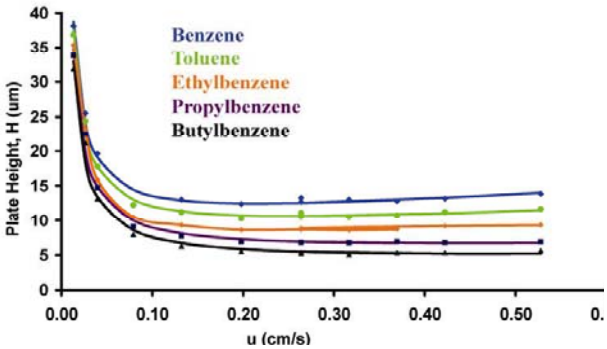

Figure 2. Plate height for Sub-2µm ZirChrom®-PBD based on van Deemter equation vs. linear velocity for alkylbenzenes; Mobile phase: 50/50 ACN/water (adjusted to keep k' constant in comparison to 3um particles)

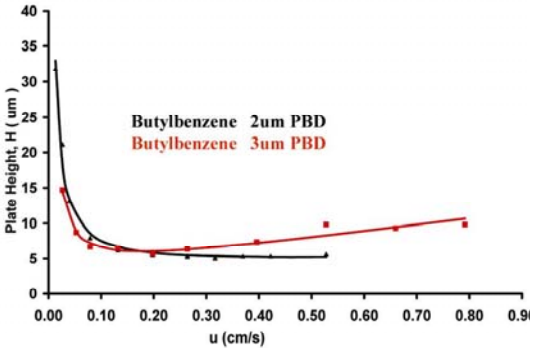

Figure 3. Plate height comparison for 3 µm and Sub-2µm ZirChrom®-PBD based on van Deemter equation vs. linear velocity for alkylbenzenes; Mobile phase: 55/45 ACN/water

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.

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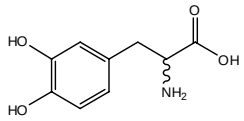
Analysis of Catecholamines on ZirChrom®-PBD

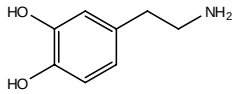
Dr. Daniel Nowlan and Kelly S. Johnson
ZirChrom Separations, Inc.

Technical Bulletin # 328

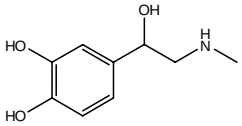
Historically, catecholamines are difficult molecules to elute on zirconia-based HPLC columns due to the strong interaction between the catechol group and the Lewis acid site dominated surface. Catecholamines are zwitterionic and are also known to be good metal chelators. However, under the right mobile phase conditions the separations of catecholamines on zirconia-based stationary phases is facile and the multi-modal surface chemistry of zirconia allows for a unique selectivity. Here we report the separation of several catecholamines and the effect of ionic strength and percent organic on the resolution of these compounds: L-dopa, tyramine, epinephrine, dopamine, and 3,4-dihydroxynorphedrine.



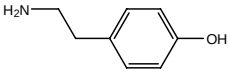
DL-Dopa



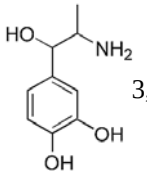
Dopamine



Epinephrine



Tyramine



3,4-dihydroxynorephedrine (DHNP)

Figure 1. Structures of Catecholamines

Introduction

Catecholamines are hormones important in causing general physiological changes that prepare the body for physical activity such as the fight or flight response. The unparalleled stability of ZirChrom®-PBD allows for a much longer column lifetime and more robust separation when compared to traditional silica-based ion-pairing techniques. The multi-modal separation capabilities of ZirChrom®-PBD allow for the unique selectivity for these ionic molecules. To achieve optimum peak shape and selectivity for these ionic molecules a mobile phase containing both a sufficient amount of Lewis base additive (phosphate), ionic strength (acetate) and organic modifier was developed. The following details the separation of five catecholamines using a multi-modal separation on a ZirChrom®-PBD column.

Experimental

Five catecholamines were separated at 35 ºC using a ZirChrom®-PBD column. The separation conditions were as follows:

Column:	ZirChrom®-PBD, 50 mm x 4.6 mm i.d. (Part Number: ZR03-0546)
Mobile Phase:	85/15 Acetonitrile/10mM Ammonium Dihydrogen Phosphate, 30mM Ammonium Acetate, pH 3.4
Temperature:	35 ºC
Flow Rate:	1.5 ml/min.
Injection Vol.:	5 µl
Detection:	UV at 254 nm

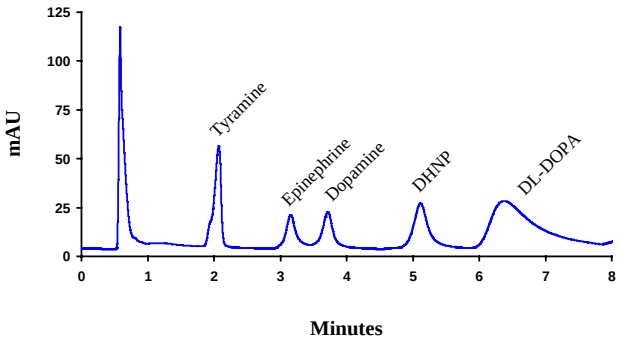


Figure 2. Separation of Catecholamines

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Comparison of Sub-2µm and 3µm ZirChrom®-PBD for the Separation of Catecholamines

Daniel Nowlan, Ph.D. and Kelly S. Johnson
ZirChrom Separations, Inc.

Technical Bulletin # 329

In this application note we examine the benefits of a smaller particle size for the analysis of catecholamines. As predicated theoretically, the decrease in particle size, from 3µm to sub-2µm, allows for a marked increase in efficiency (measured in plates/meter).

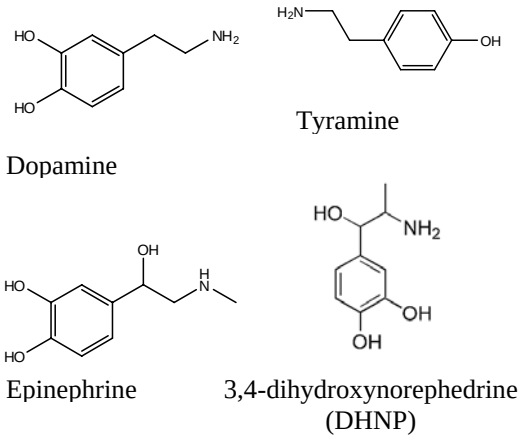

Figure 1. Structures of Catecholamines

Introduction

Catecholamines are historically difficult molecules to elute on zirconia-based HPLC columns due to the strong interaction between the catechol group and the Lewis acid site dominated surface. Previous work (1) developed mobile phase conditions that enable facile, robust and multi-modal separation of these compounds. In this application note we take the work a step further exploring the effect of particle size on the efficiency of the peaks. Theoretical calculations predict, and recent work has demonstrated, that particle size is directly proportional to column efficiency (2) & (3). This increase in efficiency is useful when requiring a bit more resolution or to speed a satisfactory separation up by utilizing smaller particles in a shorter column size.

Experimental

Four catecholamines were separated at 30 ºC using a ZirChrom®-PBD column. The separation conditions were as follows:

Columns:	Sub-2 and 3 µm ZirChrom®-PBD, 50 mm x 4.6 mm i.d. (Part Number: ZR03-0546-1.9 & ZR03-0546)
Mobile Phase:	85/15 Acetonitrile/10mM Ammonium Dihydrogen Phosphate, 30mM Ammonium Acetate, adjusted to pH 3.4 with HCl
Temperature:	30 ºC
Flow Rate:	1.5 ml/min.
Injection Vol.:	5 µl
Detection:	UV at 254 nm

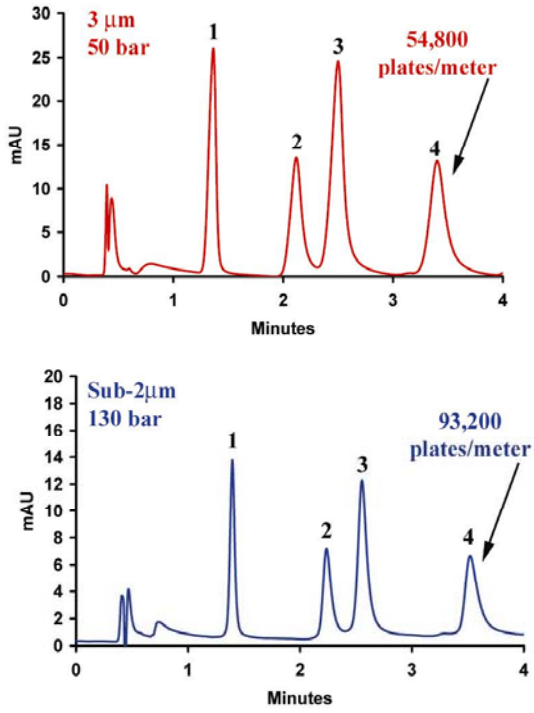

Figure 2. Separation of Catecholamines. **1**=Tyramine, **2**=Epinephrine, **3**=Dopamine, **4**=3,4-dihydroxynorephedrine

The data in Figure 2 support the hypothesis that smaller particles increase the efficiency of the column. Future work will explore the use of temperature and shorter column lengths to fully capitalize on the increased efficiency and thus resolution provided by sub-2µm particles.

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.

References

(1) http://www.zirchrom.com/pdf/328.pdf
(2) Dolan, J.W., "The Perfect Method , Part 6" LCGC Europe, February (2008).
(3)) http://www.zirchrom.com/pdf/327.pdf

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Effect of Temperature on the Analysis of Catecholamines using Sub-2µm ZirChrom®-PBD

Daniel Nowlan, Ph.D. and Kelly S. Johnson
ZirChrom Separations, Inc.

Technical Bulletin # 330

In this application note we examine the effect of temperature on a sub-2µm zirconia based phase for the analysis of catecholamines. Reducing the particle size and increasing the temperature both increases the efficiency and speed of separation without sacrificing resolution or column stability.

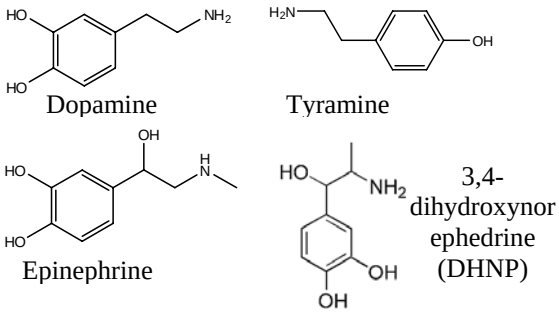

Figure 1. Structures of Catecholamines

Introduction

Catecholamines are historically difficult molecules to elute on zirconia-based HPLC columns due to the strong interaction between the catechol group and the Lewis acid site dominated surface. Previous work has been done to optimize separation conditions (1) & (2) allowing for a fast, multi-mode separation. In this application note we take the work a step further exploring the effect of temperature coupled with a smaller, sub-2µm, particle size. The unique stability of ZirChrom®-PBD enables a much wider temperature (up to 150 °C) and pH (pH 1 – 14) range for method development. Elevated temperature speeds separation through the following three main effects (3). Firstly, the viscosity of the mobile phase is decreased, enabling higher flow rates with existing equipment without increasing backpressure. Secondly, higher temperature increases the diffusion rate of analytes minimizing the any losses in efficiency at higher flow rates (4). Finally, at elevated temperature, the kinetics of the faster interactions between the analytes and stationary phase will lower the overall analysis time; often reducing or eliminating peak tailing. A decrease in mobile phase viscosity is especially important for method development with sub-2µm particles as it helps to overcome the higher back pressures inherent in small particle HPLC and allows the average user to employ these particles without the use of specialized UHPLC instrumentation.

Experimental

Four catecholamines were separated using a ZirChrom®-PBD column. The separation conditions were as follows:

Columns:	Sub-2 µm ZirChrom®-PBD, 50 mm x 4.6 mm i.d. (Part Number: ZR03-0546-1.9)
Mobile Phase:	85/15 Acetonitrile/10mM Ammonium Dihydrogen Phosphate, 30mM Ammonium Acetate, adjusted to pH 3.4 with HCl
Injection Vol.:	5 µl
Detection:	UV at 254 nm

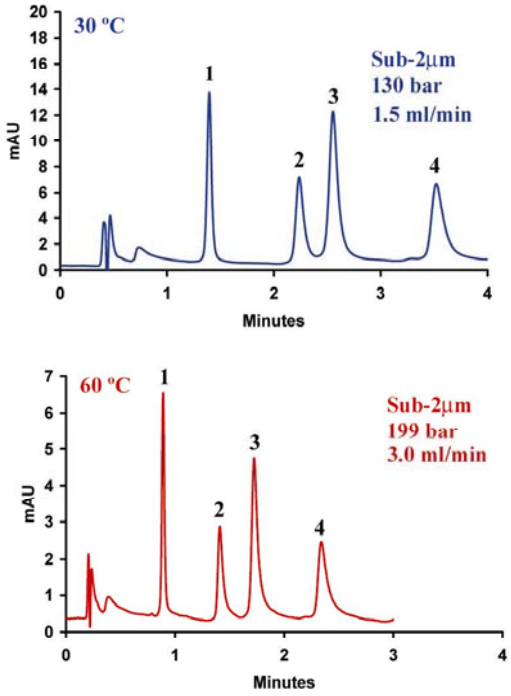

Figure 2. Separation of Catecholamines. **1**=Tyramine, **2**=Epinephrine, **3**=Dopamine, **4**=3,4-dihydroxynorephedrine

Figure 2 shows the separation of catecholamines at 30 and 60 °C on a sub-2µm ZirChrom®-PBD column. This very mild increase in temperature has allowed a two-fold increase in flow rate and has reduced the analysis time by one minute while keeping the back pressure within normal operating parameters for standard HPLC equipment.

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.

References

(1) http://www.zirchrom.com/pdf/327.pdf
(2) http://www.zirchrom.com/pdf/328.pdf
(3) Antia, F.; Horvath, C. J. Chrom. 435, 1-15 (1988)
(4) Li, J.W; Carr, P.W. Anal. Chem. 69(5), 837-843 (1997)

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High Temperature Analysis of Nine Common Pharmaceuticals using Sub-2µm ZirChrom®-PBD

Daniel Nowlan, Ph.D. and Kelly S. Johnson
ZirChrom Separations, Inc.

In this application note we examine the effect of temperature on a sub-2µm zirconia-based phase for the analysis of nine common pharmaceuticals. Reducing the particle size and increasing the temperature increases both the efficiency and speed of separation without sacrificing resolution or column stability.

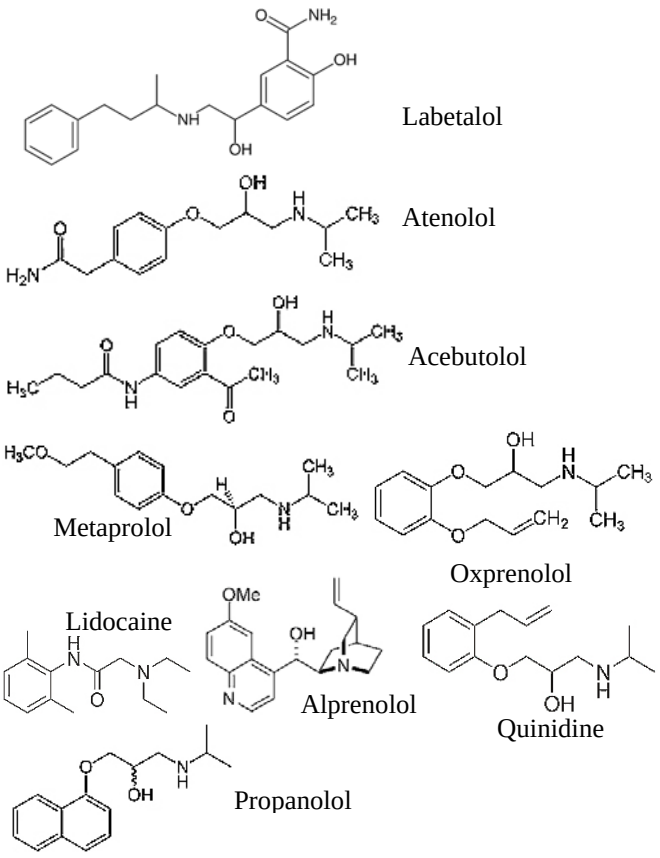

Figure 1. Structures of Pharmaceutical Compounds in Analysis

Introduction

The ability of the chromatographers to make practical use of the promised efficiency, and thus speed, benefits of sub-2µm particles has long been hindered by the need for specialized high-pressure tolerant instrumentation. The superior selectivity and stability of ZirChrom®-PBD enables a much wider temperature (up to 150 °C) and pH (pH 1 – 14) range for method development. Elevated temperature speeds separations through the following three main effects (1). First, higher temperature increases the diffusion rate of analytes minimizing any losses in efficiency at higher flow rates (2). Second, at elevated temperature, the kinetics of the faster interactions between the analytes and stationary phase will lower the overall analysis time; often reducing or eliminating peak tailing. Finally, the viscosity of the mobile phase is decreased, enabling higher flow rates with existing equipment without increasing backpressure

The decrease in mobile phase viscosity provided by high temperature is especially important for method development with sub-2µm particles as it helps to overcome the higher back pressures inherent in small particle HPLC and allows the average user to take advantage of the increased efficiency provided by the smaller particles without the use of specialized UHPLC instrumentation.

Experimental

Nine pharmaceuticals were separated using a ZirChrom®-PBD column. The separation conditions were as follows:

Columns:	Sub-2 µm ZirChrom®-PBD, 50 mm x 4.6 mm i.d. (Part Number: ZR03-0546-1.9)
Mobile Phase:	22/78 Acetonitrile/20mM Potassium Phosphate, pH 12.0
Injection Vol.:	2 µl
Temperature:	75°C
Back Pressure:	246 bar
Flow Rate:	2.5 mL/minute
Detection:	UV at 254 nm

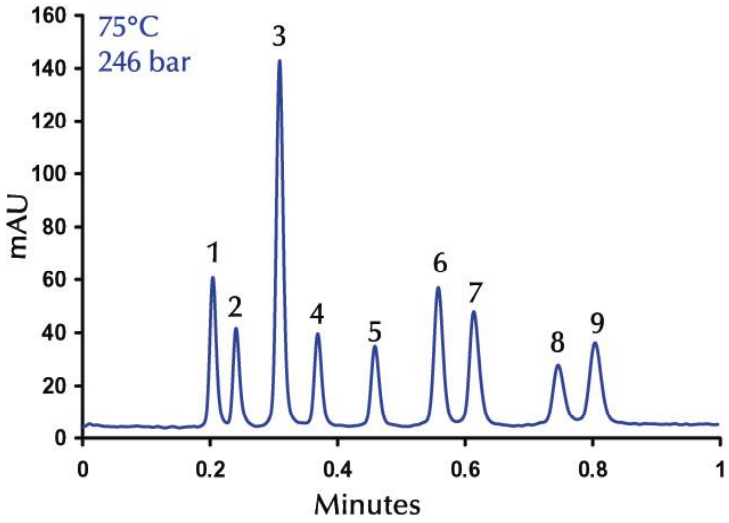

Figure 2. Separation of Nine Pharmaceuticals. **1**=Labetalol, **2**=Atenolol, **3**=Acebutolol, **4**=Metoprolol, **5**=Oxprenolol, **6**=Lidocaine, **7**=Quinidine, **8**=Alprenolol, **9**=Propranolol

Figure 2 shows the separation of nine pharmaceutical compounds at 75 °C on a sub-2µm ZirChrom®-PBD column. The instrument used in the analysis was a very basic commercially available HPLC system with minimal modification to eliminate as much void volume in the system as possible. As shown in Figure 2, the

increase in temperature allowed an increase in flow rate, reducing the analysis time while keeping back pressure well below the 400 bar operating limit for standard HPLC equipment.

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.

References

(1) Antia, F.; Horvath, C. J. Chrom. 435, 1-15 (1988)
(2) Li, J.W; Carr, P.W. Anal. Chem. 69(5), 837-843 (1997)

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Separation of Benzimidazoles and Derivatives in Anti-parasitic Drugs

Bingwen Yan, Ph.D. and Kelly Johnson
ZirChrom Separations, Inc.

Technical Bulletin # 340

This multicomponent analysis of anti-parasitic drugs achieves baseline resolution of six closely related compounds in under 20 minutes using gradient elution and the unique selectivity of ZirChrom®-PBD.

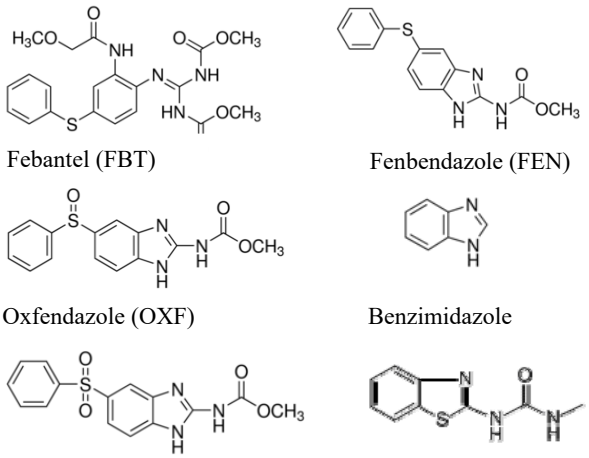

Figure 1: Structures of febantel, fenbendazole, oxfendazole, benzimidazole, oxfendazole sulfone, and benzthiazuron.

Introduction

Benzimidazoles are a large class of pharmaceuticals used in animal production with a broad spectrum of activity against roundworms (nematodes).[1] The longer half-life of oxfendazole and fenbendazole, due to their slow metabolization, allows these compounds to be more effective but also raises the concern of residues in the final food products. Here we present a gradient method of six closely related anti-parasitic pharmaceuticals.

Experimental

A mixture of six anti-parasitic pharmaceuticals was separated at 30 ºC using a ZirChrom®-PBD column. Samples were 1 mg/mL and were in acetonitrile with the exception of benzthiazuron which was in methanol. The separation conditions were as follows:

Column: ZirChrom®-PBD, size 150 mm x 4.6 mm i.d. (Part Number: ZR03-1546)

Mobile Phase: A: 15mM Ammonium Formate, pH 7.6
B: Acetonitrile

Time (min.)	% A	% B
0	96	4
8	96	4
20	80	20
25	96	4

Temperature: 30 ºC
Flow Rate: 1 ml/min.
Injection Vol.: 5 µl
Pressure Drop: 195 bar
Detection: UV at 254 nm

In Figure 2 the superior multi-modal selectivity of ZirChrom®-PBD for these compounds is demonstrated. All six components are resolved. The additional peaks at approximately 13 and 20 minutes are believed to be artifacts from the gradient.

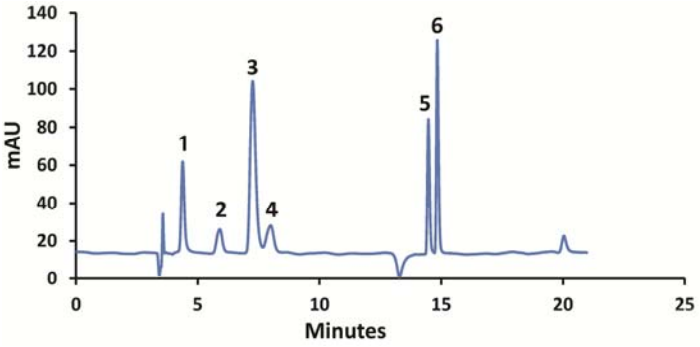

Figure 2: 1=Benzimidazole, 2=Oxfendazole, 3=Benzthiazuron , 4=Oxfendazole Sulfone, 5=Fenbendazole, 6=Fenbentel

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)http://www.merckvetmanual.com/mvm/pharmacology/anthelmintics/benzimidazoles.html

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