ZirChrom®

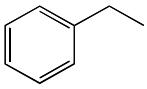
Technical
Bulletin #134

... For Peak Performance

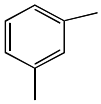
Isomer Separation on ZirChrom®-CARB

Analytes

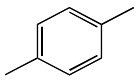
1 - Ethylbenzene



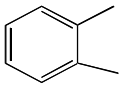
2 - m-Xylene

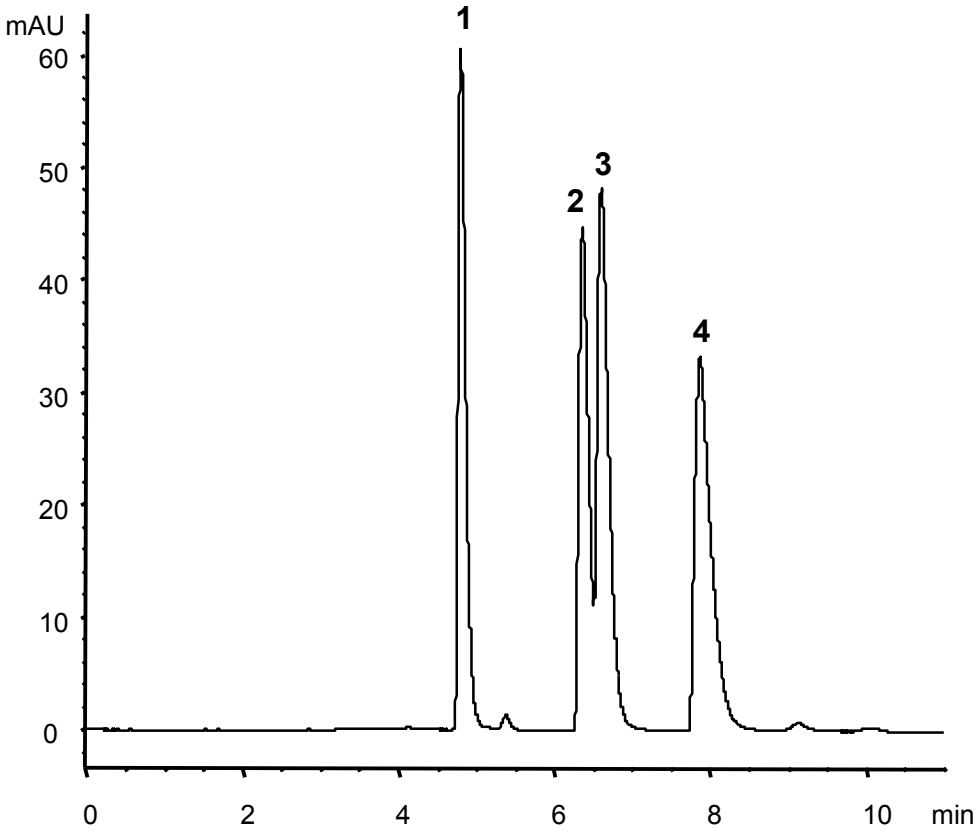


3 - p-Xylene



4 - O-Xylene





LC Conditions

Column: A: ZirChrom®-CARB, 150 × 4.6 mm;
Mobile Phase: 50/50 A/B
A: Acetonitrile
B: Water

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

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Technical
Bulletin #203

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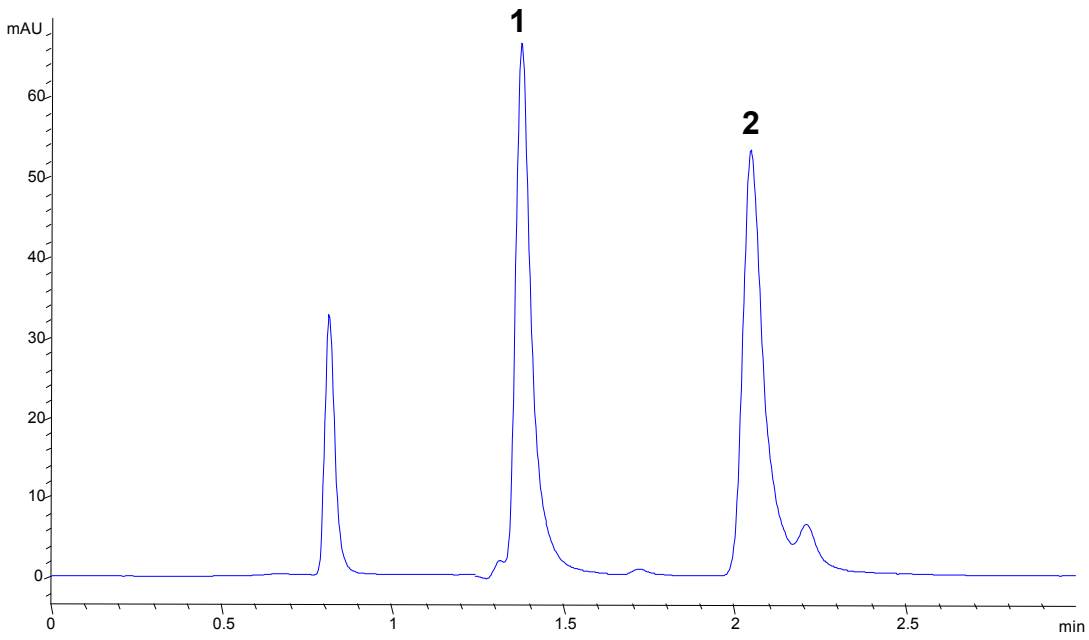
Cosmetics on ZirChrom®-CARB

Analytes

1 - Allantoin
2 - Bronopol



LC Conditions

Column: ZirChrom®-CARB, 100 × 4.6 mm
Mobile Phase: 20/80 A/B
A: Acetonitrile
B: Water

Flow rate: 1.0 mL/min.
Temperature: 25 ºC
Detection: 200 nm

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Technical Bulletin #244

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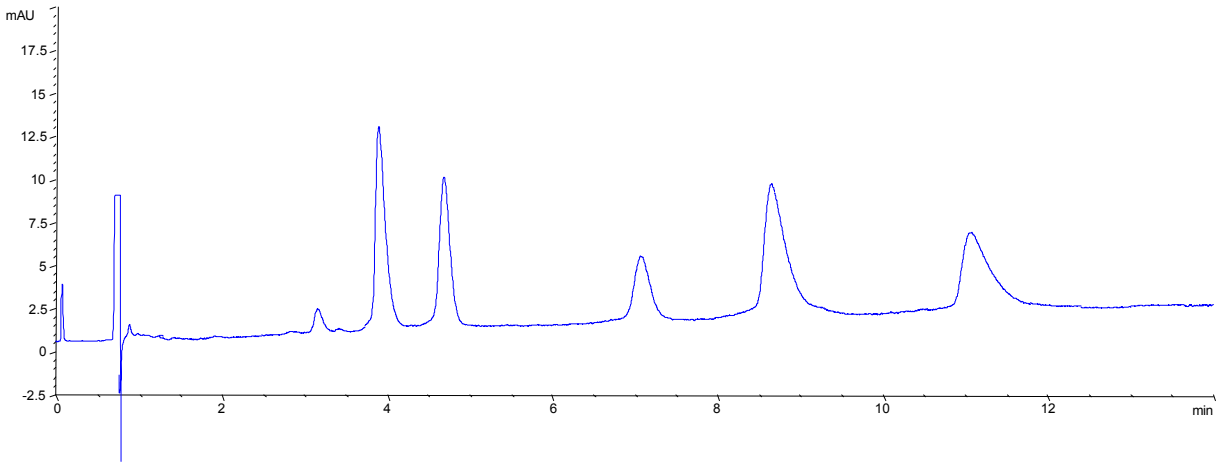
Separation of a Steroid and its Metabolites on ZirChrom®-CARB



Compound	T_R	Area	Height	k'	a [n/(n-1)]	Tailing Factor	Calc. Plates	Calc. Rs
A	3.371	14.1	2.6	1.86	N/A	1.47	8736	N/A
B	4.505	87.2	8.6	2.83	1.52	2.38	4463	4.20
C	5.247	72.6	8.8	3.46	1.22	1.54	9145	3.38
D	7.661	55.8	4.7	5.51	1.59	1.54	9414	7.64
E	9.955	125.9	6.2	7.46	1.35	1.92	5433	4.25
F	11.542	75.5	3.7	8.81	1.18	1.72	7233	2.92

LC Conditions

Column: ZirChrom®-CARB, 100 x 4.6 mm
Mobile Phase: 20/30/30/20 A/B/C/D
A: ACN
B: MeOH
C: THF
D: Water

Flow rate: 1.0 mL/min.
Temperature: 60 ºC
Injection volume: 5 µL
Detection: 200 nm

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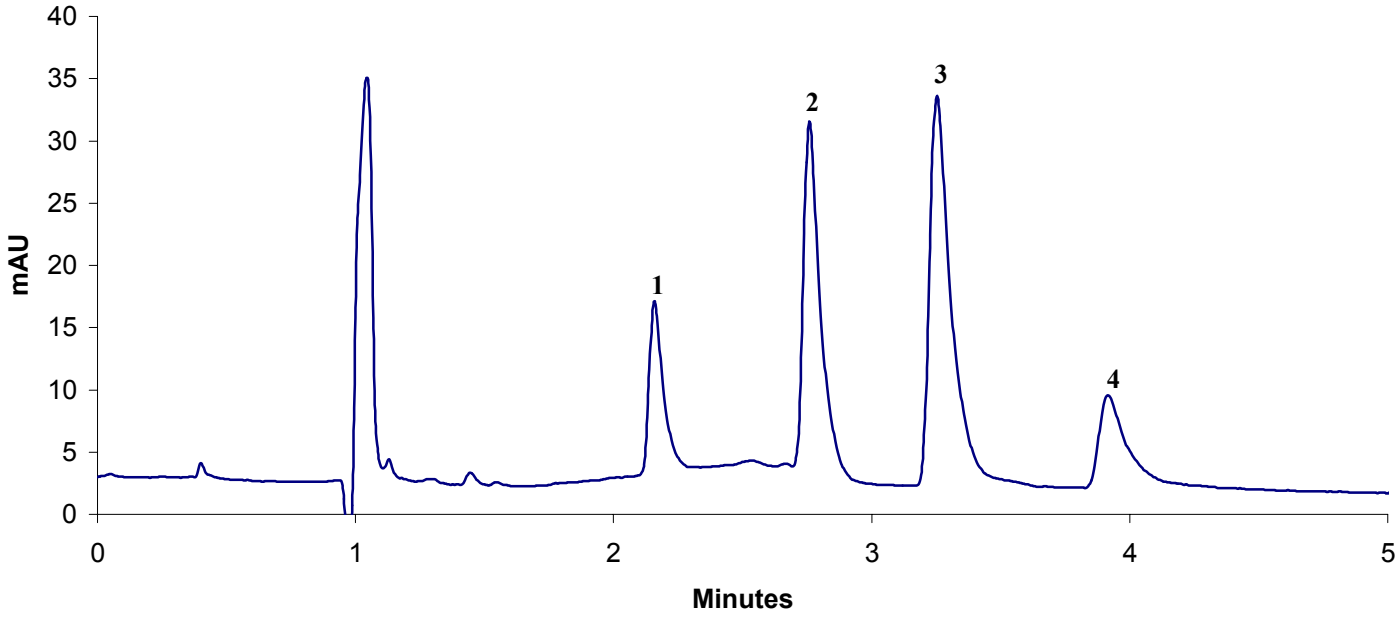
Technical Bulletin #265

... For Peak Performance

Separation of Corticosteroids on ZirChrom®-CARB



Analytes*

1 - Dexamethasone, 2 - Prednisone, 3 - Prednisolone, 4 - Betamethasone

**Peak assignments revised 09-04-03*

LC Conditions

Column: ZirChrom®-CARB, 150 mm x 4.6 mm i.d., (part# ZRO1-1546)
Mobile Phase: 60/10/30 A/B/C
A: ACN
B: MTBE
C: Water

Flow rate: 1.5 mL/min.
Temperature: 80 ºC
Injection volume: 15 µL
Detection: 215 nm
Pressure Drop: 160 bar

Fast Methods for Structurally Similar Compounds, Carbon HPLC Columns
Technical Bulletin #266

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

Method development can be challenging for structurally similar compounds in RPLC. A new carbon-clad zirconia phase offers dramatically different chromatographic selectivity for these types of compounds when compared to traditional silica-based bonded phases. This note shows baseline separation of 6 structurally similar sulfate-steroid conjugates using a zirconia-based ZirChrom-CARB HPLC column.

Introduction

Method development in reversed-phase liquid chromatography (RPLC) has traditionally been difficult for molecules which are geometric isomers or structurally very similar. In bonded-phase silicas, the partition mechanism responsible for retention in RPLC often does not offer adequate chemical selectivity for such compounds. On the other hand, carbon-based phases, provide retention in RPLC through an adsorption mechanism which often times increases the chromatographic selectivity for these types of compounds and dramatically increases the chances of resolving pairs of these analytes. In addition to this enhanced chromatographic selectivity, carbon-based phases also offer increased chemical and stability of the stationary phase.

Carbon-clad zirconia

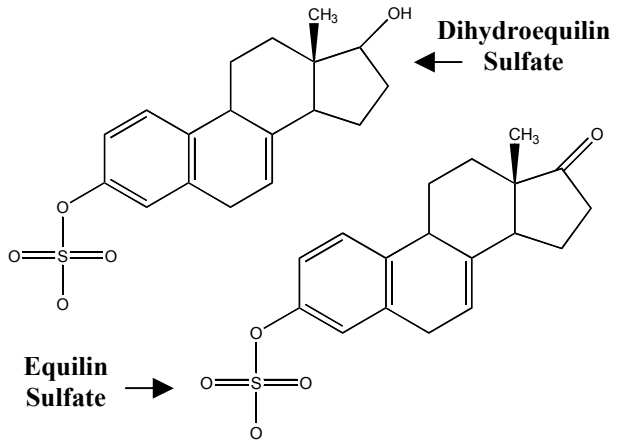
Zichrom Separations, Inc. has developed new materials using zirconia as a stationary phase support, and patented chemical vapor deposition technology to produce a carbon-clad zirconia particle suitable for use in reversed-phase liquid chromatography. The surface bond is C-C, which is extremely resistant to chemical and thermal attack. The authors have run mobile phases at very high pH (1M NaOH), very low pH (0.5M HNO_3), and at elevated temperature (up to 150 ^oC), and have not observed loss of stationary phase.

Experimental

A mixture of sulfate-steroid conjugates was separated at elevated temperature using a ZirChrom®-CARB column. The separation conditions were as follows:

Column:	4.6 mm x 100 mm ZirChrom-CARB
Mobile Phase:	Gradient elution from 55/5/40 to 90/5/5 A/B/C from 0 to 4.5 minutes. A: Acetonitrile B: Tetrahydrofuran C: 25mM Ammonium fluoride, 10mM Ammonium acetate, pH 5.6
Temperature:	80 ^oC
Injection Vol.:	10 µl
Flow rate:	3.0 ml/min.
Pressure Drop:	195 bar
Detection:	UV at 270 nm

Figure 1 – Structures of steroid-sulfate Conjugates



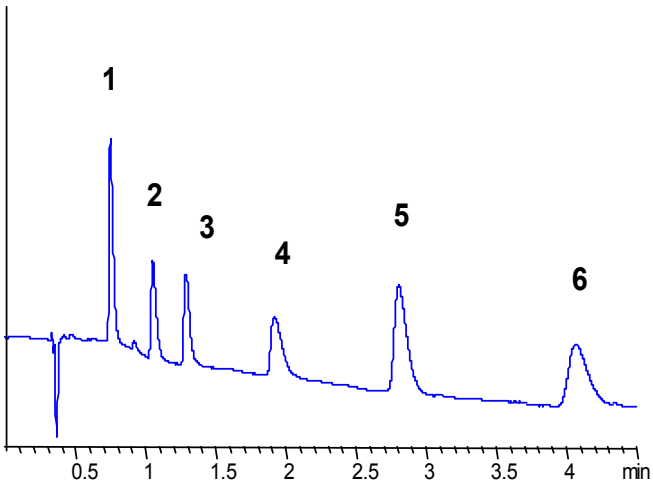


Figure 2: Steroid conjugate selectivity
1=Dihydroestradiol sulfate, 2=Dihydroequilin sulfate, 3=Equilin sulfate, 4=Estrone sulfate, 5=Equilenin sulfate, 6=Dihydroequilenin sulfate

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Technical Bulletin #273

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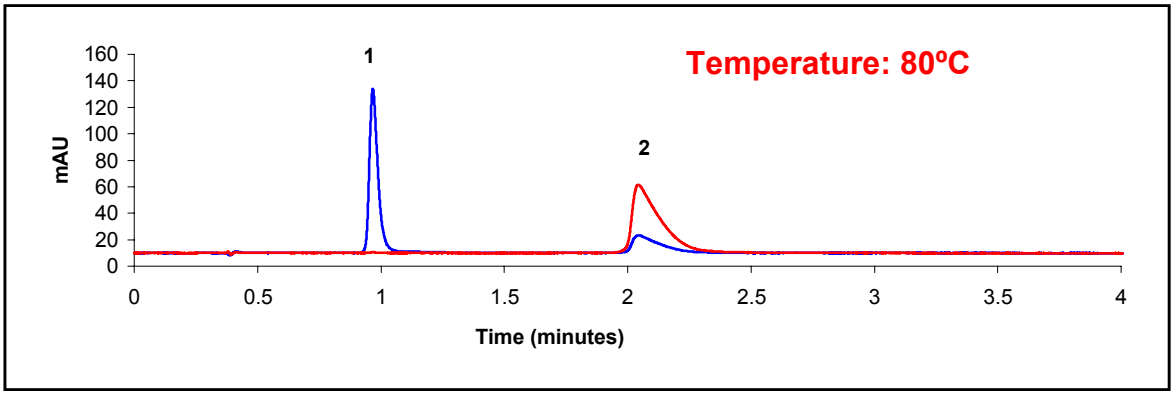
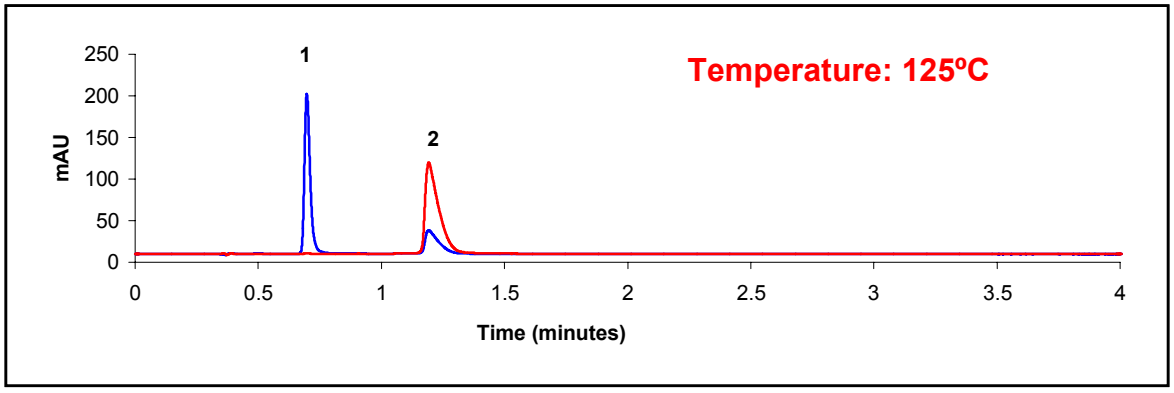
Separation of Reproductive Steroids on ZirChrom®-CARB





Column: ZirChrom®-CARB 50mm × 4.6 mm i.d.
Mobile Phase: Isocratic 42.5/42.5/15 A/B/C

A: Acetonitrile
B: 1-Butanol
C: Water

Temperature: 80ºC and 125ºC with Metalox 200-C

Detection: Blue-242nm Red-280 nm
Injection: 1 uL
Solutes: 1. Testosterone
2. Estradiol
Flow Rate: 1.5 ml/min

Fast Separation of Eleven Nitroaromatic Compounds on ZirChrom®-CARB

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

Technical Bulletin #280

This technical bulletin details the separation of eleven closely related nitroaromatic compounds, namely RDX, HMX, Nitrobenzene, 2-Nitrotoluene, Tetryl, 2,6-Dinitrotoluene, 4-Nitrotoluene, 1,3-Dinitrobenzene, 2,4-Dinitrotoluene, 2-amino 4,6-dinitrotoluene, 1,3,5-Trinitrobenzene. Our customers have reported that similar separations on silica-based phases produce run times as long as thirty minutes. Here we report a method on ZirChrom®-CARB at a column temperature of 125ºC in under 4 minutes using a Metalox™ 200-C heater.

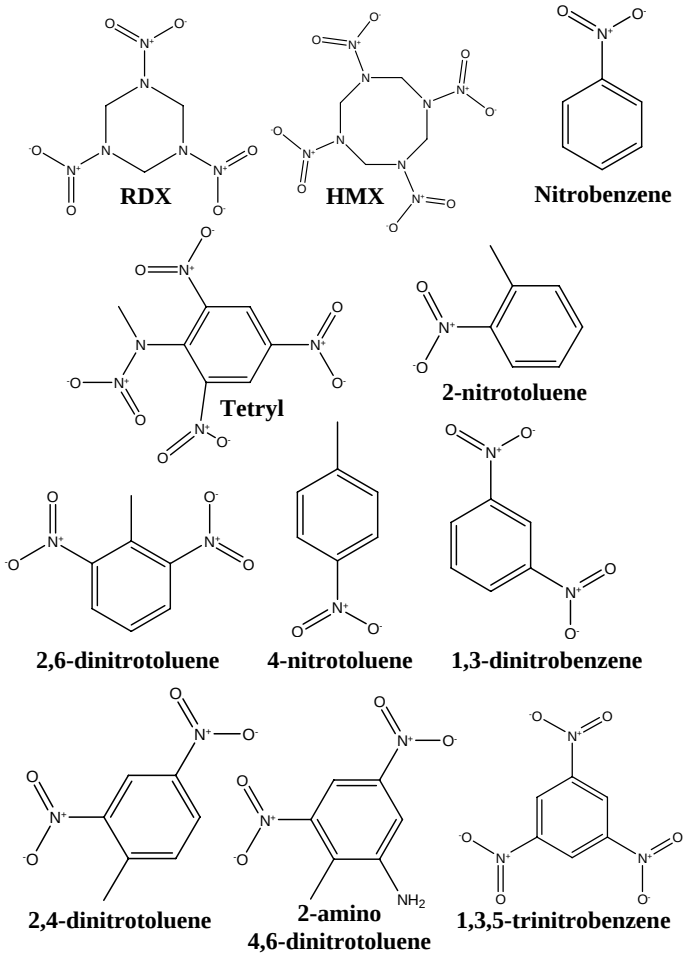


Figure 1: Structures of Explosives

Introduction

The rapid and accurate detection of nitroaromatic compounds is difficult due to the structurally similarity of the compounds (see Figure 1). A new method developed at ZirChrom employs the unique temperature stability and surface chemistry of zirconia to achieve baseline resolution of these compounds in less than 4 minutes.

Experimental

A mixture of nitroaromatics was separated at 125ºC using a ZirChrom®-CARB column (See Figure 2). The separation conditions were as follows:

Column:	ZirChrom®-CARB, 150mm x 4.6mm i.d., (part#ZR01-1546)
Mobile phase:	Isocratic Pre-mixed 35/15/50 Acetonitrile/Tetrahydrofuran/20mM ammonium carbonate pH 5.7, 10mM octylamine
Flow Rate:	2.0 ml/min.
Temperature:	125ºC (Metalox™ 200-C Heater)
Detection:	254 nm
Inj. Volume:	1µl

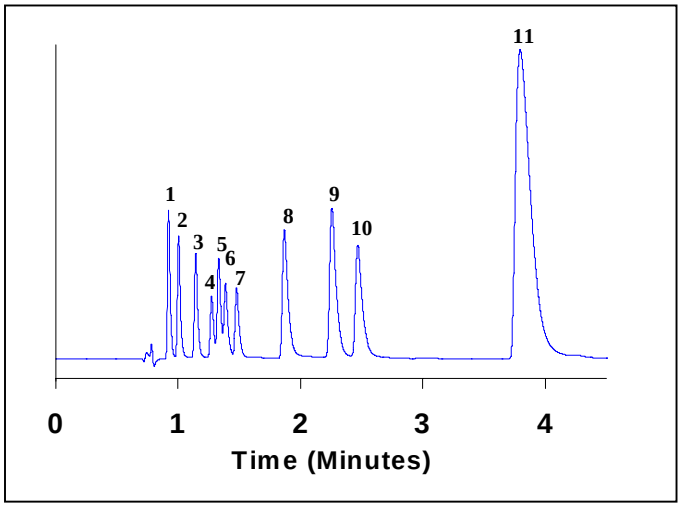


Figure 2: Separation of eleven nitroaromatics on ZirChrom®-CARB. 1=RDX, 2=HMX, 3=Nitrobenzene, 4=2-Nitrotoluene, 5=Tetryl, 6=2,6-Dinitrotoluene, 7=4-Nitrotoluene, 8=1,3-Dinitrobenzene, 9=2,4-Dinitrotoluene, 10=2-amino 4,6-dinitrotoluene, 11=1,3,5-Trinitrobenzene

This separation allows for clear identification and quantification of these compounds without the use of expensive MS detection. The separation is also completed using isocratic conditions, thus facilitating a more reproducible transfer from LC to LC.

Acknowledgments

We would like to thank Richard Burrows, Severn Trent Laboratories, for his many helpful discussions.

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Fast Separation of Acrylamide Monomer from Acrylic Acid

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

Technical Bulletin #281

The unique selectivity, pH and temperature stability of the ZirChrom®-CARB phase allows baseline resolution of acrylamide and acrylic acid using a 4 minute gradient method.

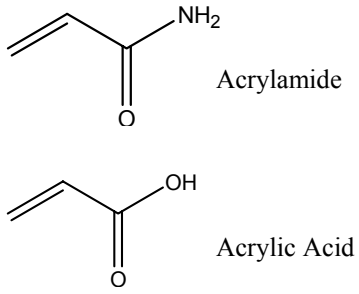

Figure 1: Structures of acrylamide and acrylic acid

Introduction

Small polar molecules such as acrylamide and acrylic acid, (see figure 1), can be difficult if not impossible to separate on conventional reversed-phase bonded silica type phases using HPLC. Acrylamide historically has been analyzed using sample derivitization coupled with gas chromatography (1). The distinctive selectivity and increased retentiveness of the ZirChrom®-CARB phase, compared to bonded silica or polymeric phases, enables the baseline resolution of these small polar molecules.

Experimental

A mixture of acrylamide monomer and acrylic acid was separated using ZirChrom®-CARB.

Column: ZirChrom®-CARB 50 mm x 4.6 mm i.d.
Column Part #: ZR01-0546
Mobile Phase: Gradient elution
A: Acetonitrile
B: 25mM phosphoric acid, pH 1.9

Time	% A	%B
0	0	100
4	25	75

Injection Vol.: 1 µl
Pressure Drop: 180 bar
Detection: UV at 210 nm
Temperature: 40 ºC with Metalox® 200-C
Flow Rate: 2 ml/min

This method is an excellent example of how the enlarged zirconia method development "tool box" can be used to overcome the toughest separation challenges. Using temperature, extreme pH and the unique surface chemistry of ZirChrom®-CARB a fast separation of acrylamide and acrylic acid was achieved using a 4 minute gradient (see figure 2).

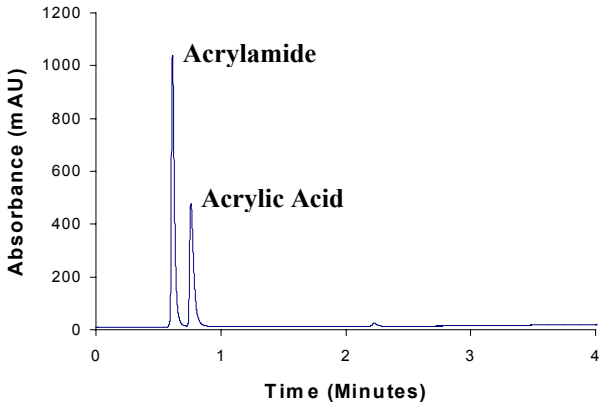

Figure 2: Separation of acrylamide and acrylic acid at 40 ºC using a ZirChrom®-CARB column.

This method can be tailored to your specific application needs. ZirChrom method developers can help you optimize and transfer this method to your site. Please contact ZirChrom technical support at 1-866-STABLE1 or support@zirchrom.com for details.

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

References

(1) E. Tareke, et al., "Acrylamide: A Cooking Carcinogen?" Chem. Red. Toxicol. 2000, 12, 517-522

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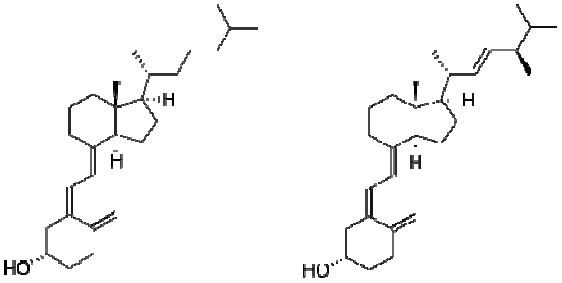
Fast Resolution of Vitamin D_2 from Vitamin D_3 on ZirChrom®-CARB

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

Technical Bulletin # 338

In this application we examine the superior selectivity of the ZirChrom®-CARB phase for two closely related compounds; vitamin D_2 and D_3.



Cholecalciferol Ergocalciferol

Figure 1: Structures of cholecalciferol (vitamin D_3) and ergocalciferol (vitamin D_2).

Introduction

Vitamin D refers to a group of fat-soluble secosteroids which are critical for enhancing intestinal absorption of important nutients. The most biologically relevant members of this group for humans are ergocalciferol (vitamin D_2) and cholecalciferol (vitamin D_3).

These closely related compounds are difficult to separate quickly using traditional reversed phase columns. Additionally, these highly reactive compounds are difficult to quantify precisely using LC/MS as the ionization process causing instability, lowing the robustness of the method (1).

Here we present an isocratic method that provides baseline resolution of a mix of vitamin D_2 and D_3 standards in two minutes using a ZirChrom®-CARB phase with UV detection at 275 nm.

Experimental

A mixture of two standards, cholecalciferol and ergocalciferol, was separated at 70 ºC temperature using a ZirChrom®-CARB column. The separation conditions were as follows:

Column:	ZirChrom®-CARB, 50 mm x 4.6 mm i.d. (Part Number: ZR01-5046)
Mobile Phase:	A: 50/50 ACN/IPA B: THF
Temperature:	70 ºC
Flow Rate:	1.5 ml/min.
Injection Vol.:	5 µl
Pressure Drop:	74 bar
Detection:	UV at 275 nm

ZirChrom®-CARB separation of cholecalciferol and egocalciferol allows for baseline resolution of the compounds in two minutes using isocratic conditions and UV detection.

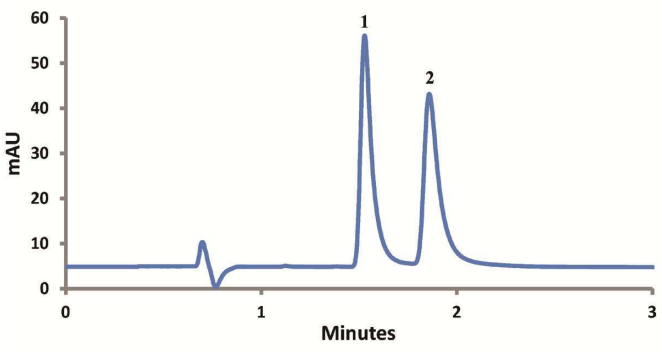


Figure 2: 1 = Cholecalciferol (vitamin D_3), 2 = Egocalciferol (vitamin D_2)

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) Brydwell, C.W. et al, Am J Clin Nutr, Vol. 88 no.2, 5545-5575 (2008)

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