ZirChrom®

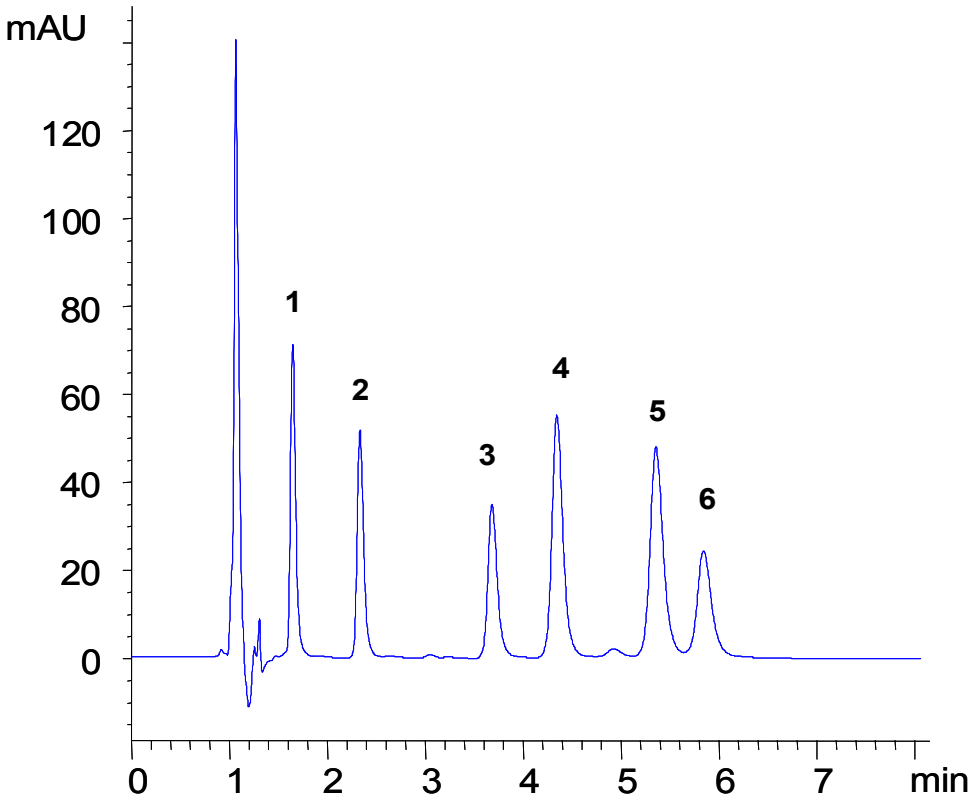
Technical
Bulletin #222

... For Peak Performance

Separation of Barbiturates

Analytes

1 - Barbital
2 - Metharbital
3 - Butethal
4 - Hexobarbital
5 - Mephobarbital
6 - Pentobarbital



LC Conditions

Column: DIAMOND BOND™ C18, 100 × 4.6 mm
Mobile Phase: 15/10/75 A/B/C
A: ACN
B: THF
C: 20 mM Ammonium phosphate, pH 7.0

Flow rate:	1.0 mL/min.
Temperature:	30 ºC
Injection volume:	5 µL
Detection:	254 nm

ZirChrom®

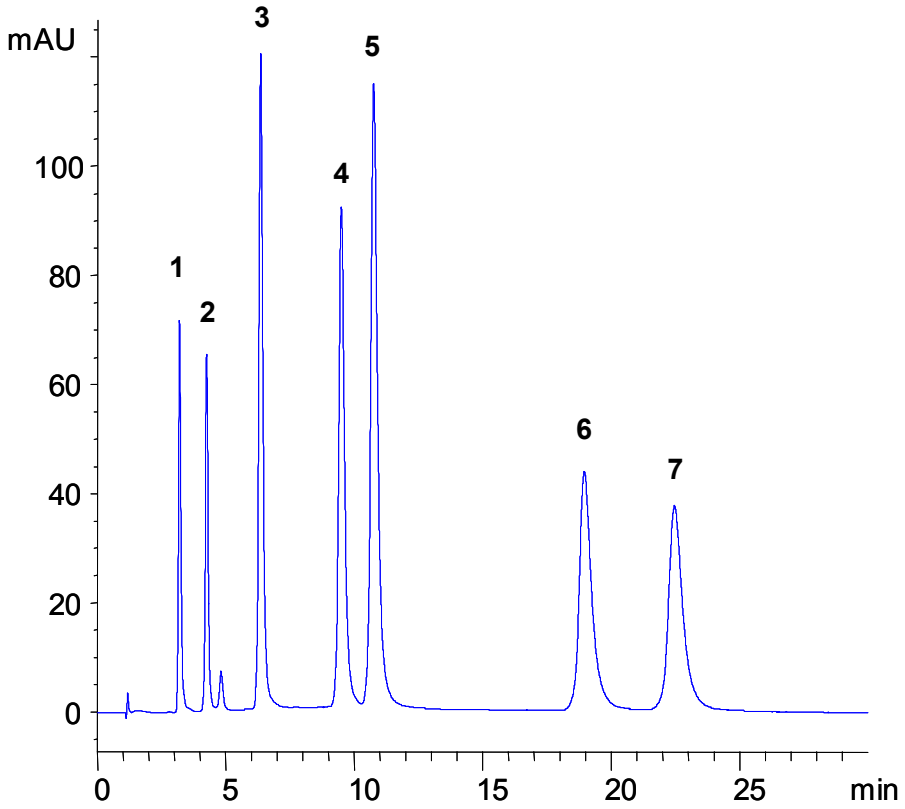
Technical
Bulletin #223

... For Peak Performance

Separation of PTH-Amino Acids

Analytes

1 - Serine
2 - Glycine
3 - Alanine
4 - Aminoisobutyric Acid
5 - Aminobutyric Acid
6 - Valine
7 - Norvaline



LC Conditions

Column: DIAMONDBOND™ C18, 100 × 4.6 mm
Mobile Phase: 20/80 A/B
A: ACN
B: 0.1% Trifluoroacidic Acid, pH 1.9

Flow rate: 1.0 mL/min.
Temperature: 40 ºC
Injection volume: 5 µL
Detection: 254 nm

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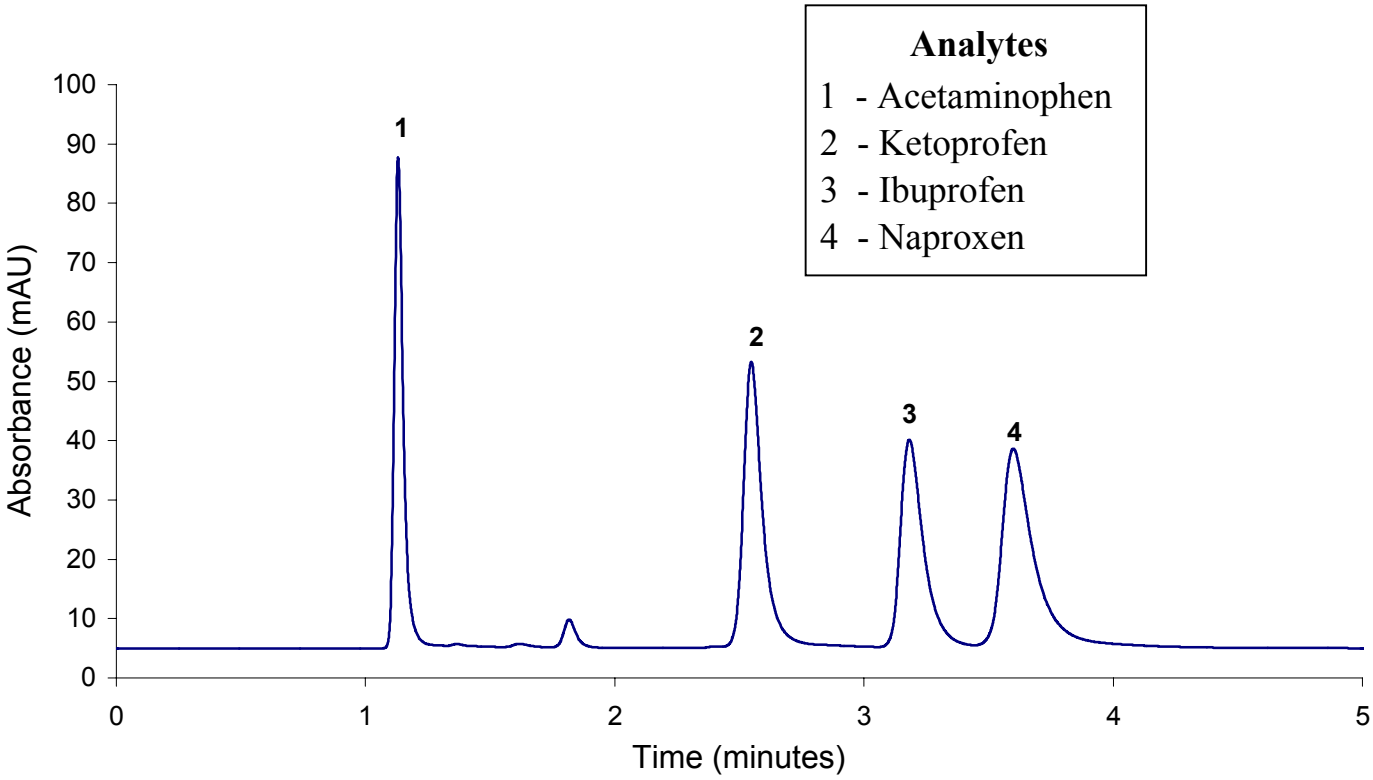
Technical
Bulletin #224

... For Peak Performance

Separation of NSAIDs



LC Conditions

Column: DIAMOND BOND™ C18, 100 mm × 4.6 mm i.d.
Mobile Phase: 50/50 A/B
A: Acetonitrile
B: Water with 50 mM Phosphoric acid at pH 1.75
Flow rate: 1.0 mL/min.
Temperature: 65 ºC
Injection volume: 1 µL

ZirChrom®

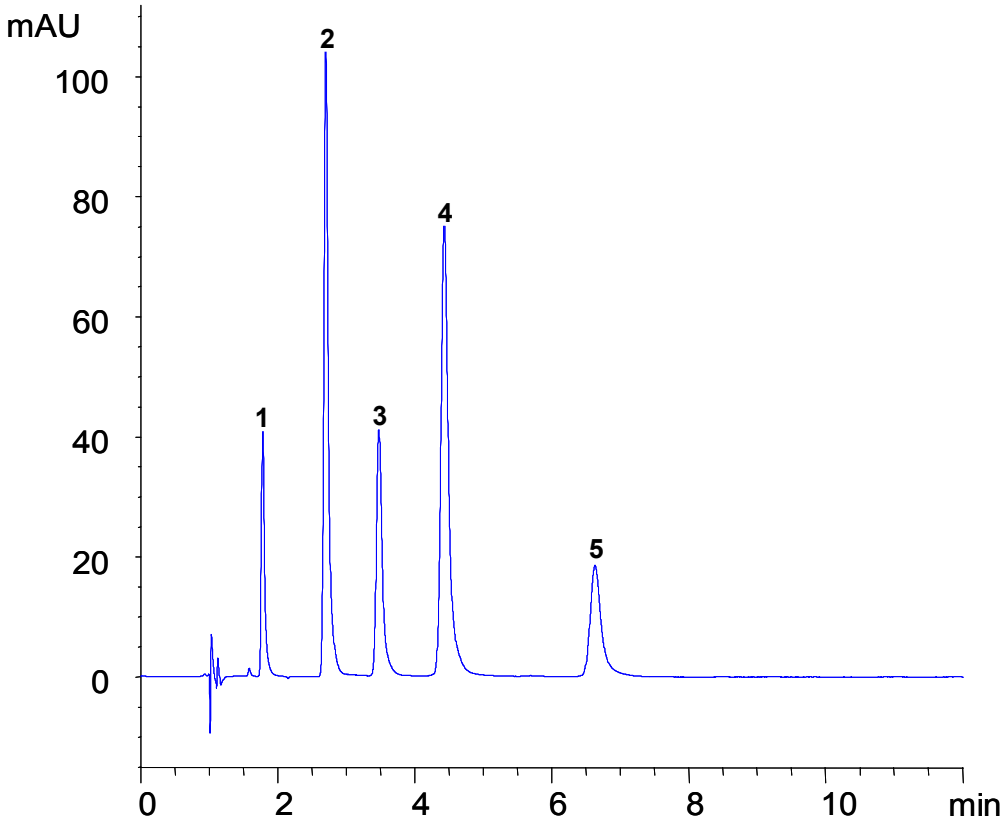
Technical
Bulletin #226

... For Peak Performance

Separation of Anticonvulsants

Analytes

1 - Primidone
2 - Metharbital
3 - Mephenytoin
4 - Phenobarbital
5 - Phenytoin



LC Conditions

Column: DIAMONDBOND™ C18, 100 × 4.6 mm
Mobile Phase: 25/75 A/B
A: THF
B: 50 mM Ammonium phosphate, pH 7.0

Flow rate:	1.0 mL/min.
Temperature:	30 ºC
Injection volume:	0.5 µL
Detection:	220 nm

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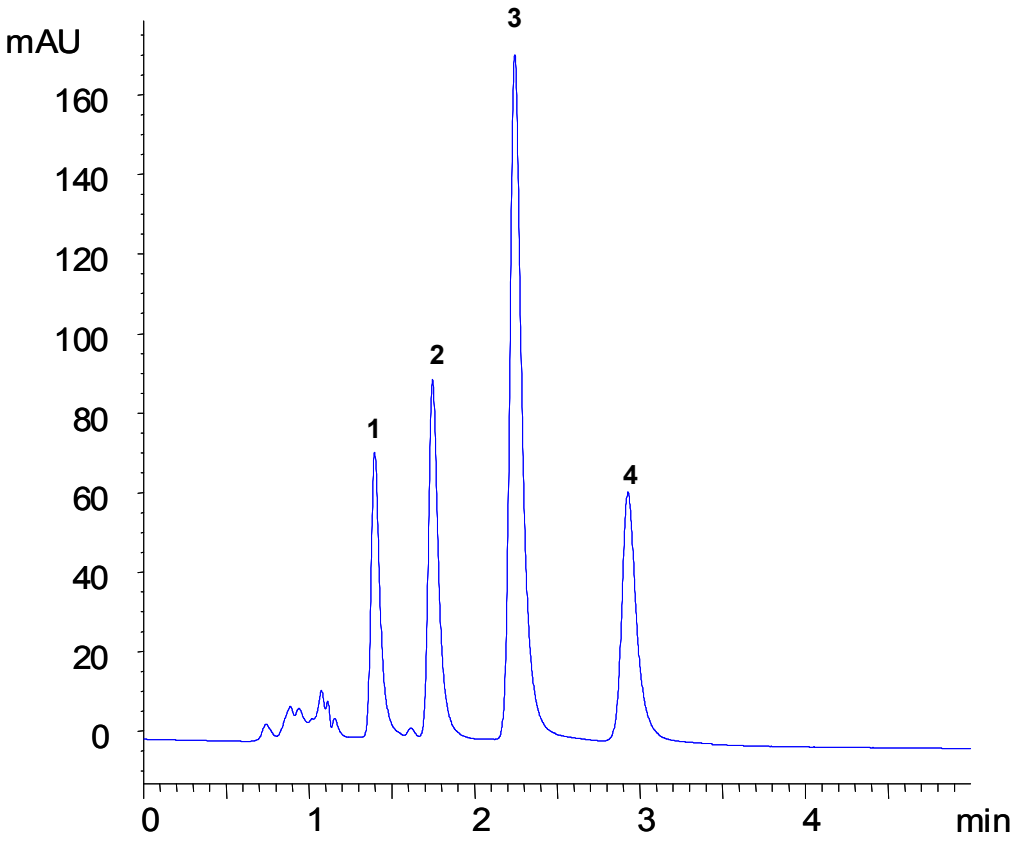
Technical
Bulletin #231

... For Peak Performance

Separation of Opioids

Analytes

1 - Naloxone
2 - Codeine
3 - Ethylmorphine
4 - Oxycodone



LC Conditions

Column: DIAMOND BOND™ C18, 100 × 4.6 mm
Mobile Phase: 26.5/73.5 A/B
A: THF
B: 20 mM Ammonium phosphate, pH 11.0

Flow rate:	1.0 mL/min.
Temperature:	40 ºC
Injection volume:	1 µL
Detection:	220 nm

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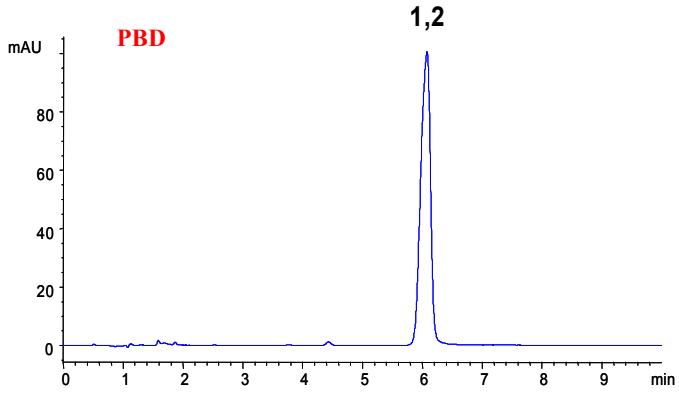
Technical Bulletin #232

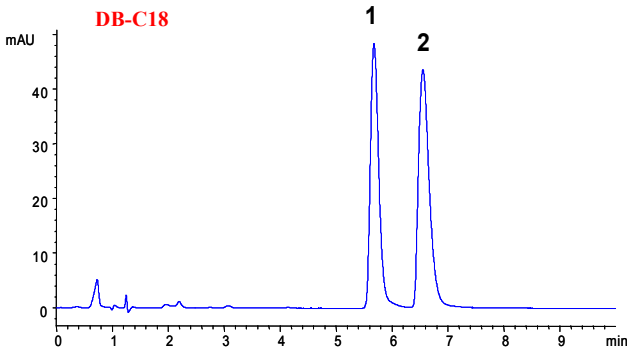
... For Peak Performance

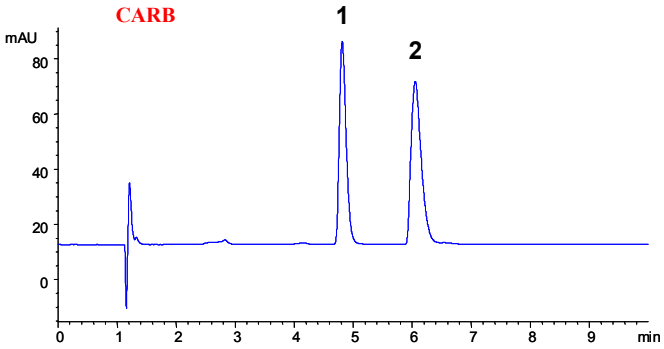
Selectivity Comparison: PBD, DB-C18 & CARB

Analytes

1 - Ethylbenzene
2 - p-Xylene







LC Conditions

Column: PBD, DIAMONDBOND™ C18, CARB, 100 × 4.6 mm
Mobile Phase: (PBD) 35/65, (DB-C18) 37.5/57.5/5, (CARB) 32.5/67.5 A/B/C

A: ACN
B: Water
C: THF

Flow rate: 1.0 mL/min.
Temperature: 30 ºC (PBD)
60 ºC (DB-C18)
60 ºC (CARB)
Injection: 5 µL
Detection: 254 nm

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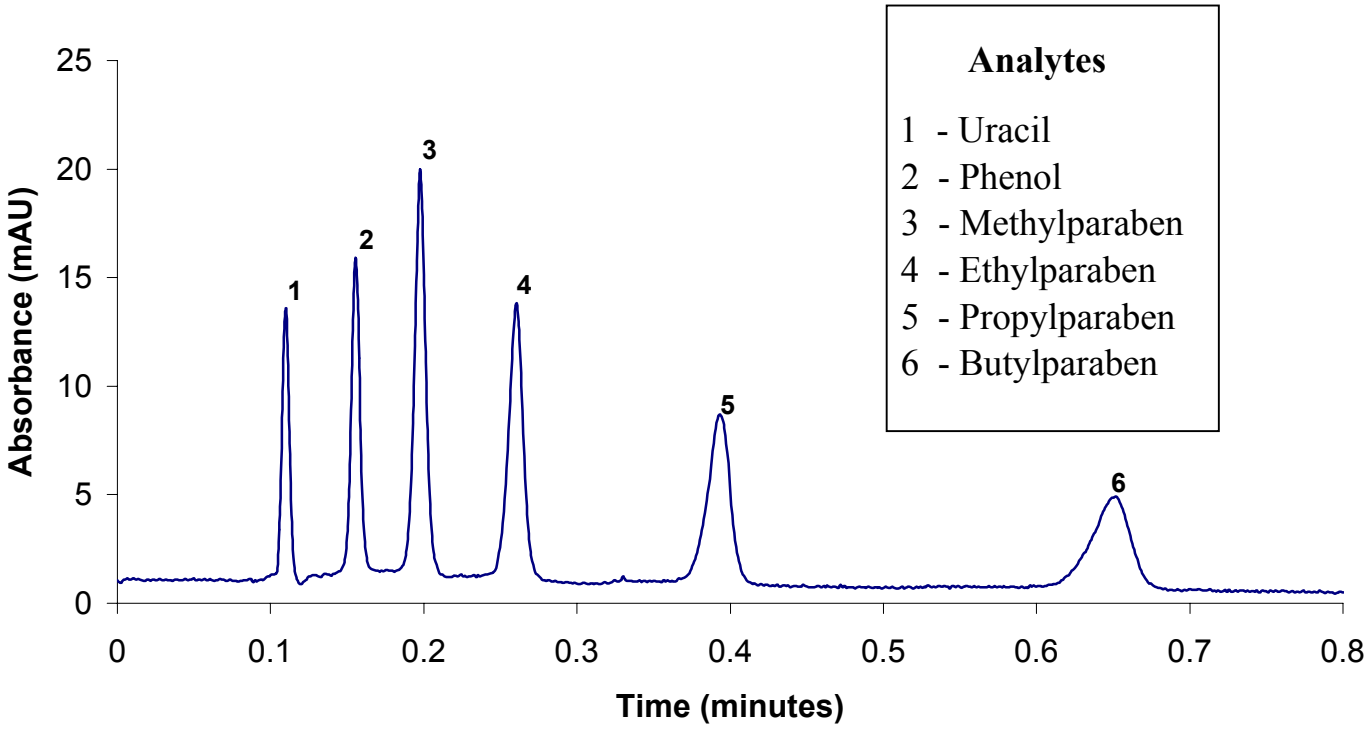
Technical Bulletin #235

... For Peak Performance

Parabens and Phenols Separation at 150 ºC on Diamondbond™-C18



LC Conditions

Column: DIAMONDBOND™ C18, 50 mm× 4.6 mm i.d.

Mobile Phase: 20/80 A/B

A: ACN

B: 20 mM phosphoric acid, pH 2.3

Flow rate:	5.5 mL/min.
Temperature:	150 ºC
Injection volume:	1 µL
Detection:	254 nm

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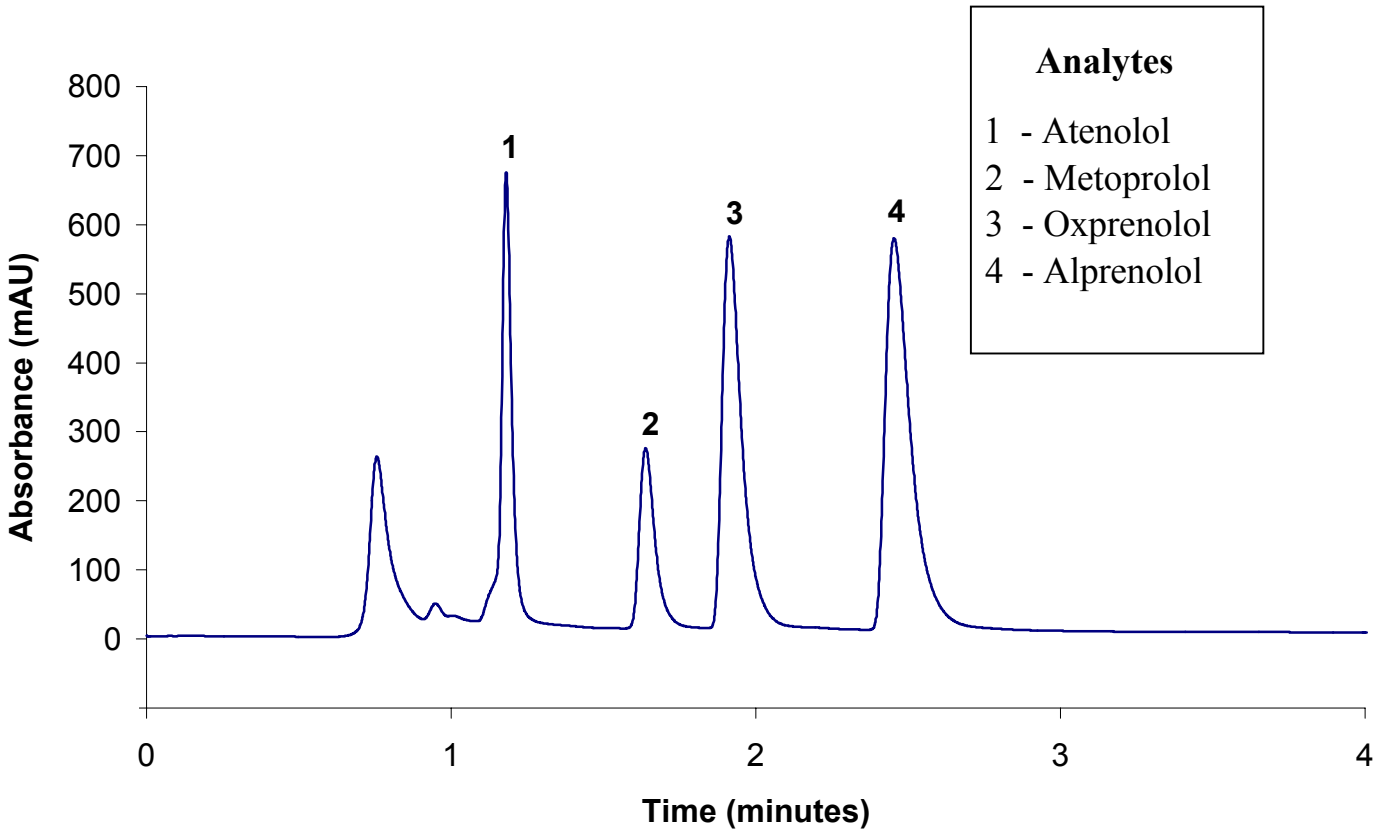
Technical Bulletin #236

... For Peak Performance

Separation of Beta-Blockers



LC Conditions

Column: DIAMOND BOND™ C18, 100 × 4.6 mm

Mobile Phase: 20/20/60 A/B/C

A: ACN

B: THF

C: 20 mM Ammonium phosphate, pH 11.0

Flow rate:	1.0 mL/min.
Temperature:	75 ºC
Injection volume:	5 µL
Detection:	254 nm

ZirChrom®

Technical
Bulletin #238

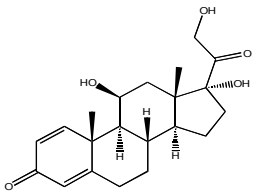
... For Peak Performance

Improved Steroid Hydrocarbon Selectivity on DiamondBond™-C18

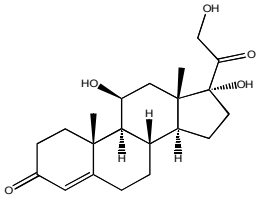
Note: Prednisolone differs from Hydrocortisone only by one hydrocarbon double bond.

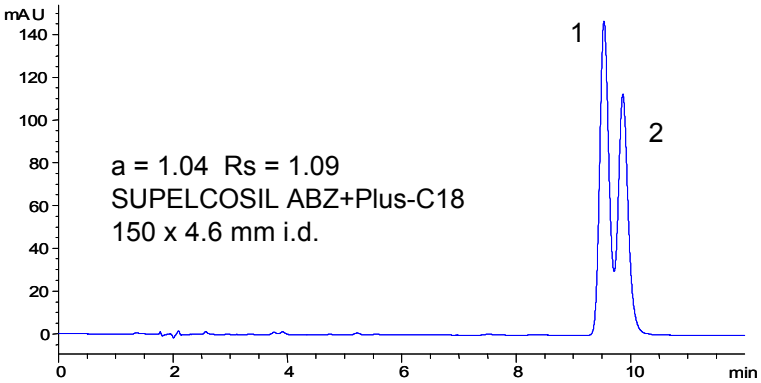
Analytes

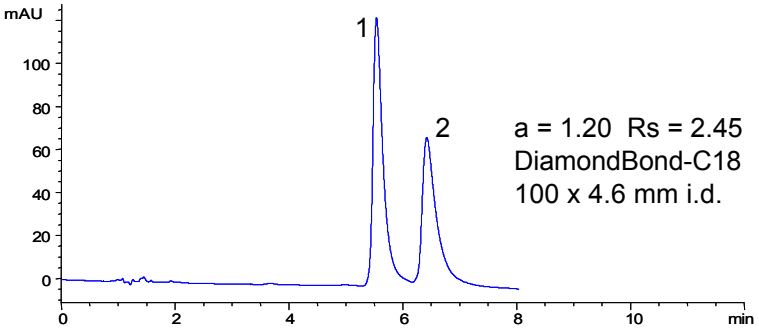
1 - Prednisolone



2 - Hydrocortisone







LC Conditions

Mobile Phase: 10/10/80 A/B/C
A: ACN
B: THF
C: 25 mM Potassium phosphate, pH 7.0

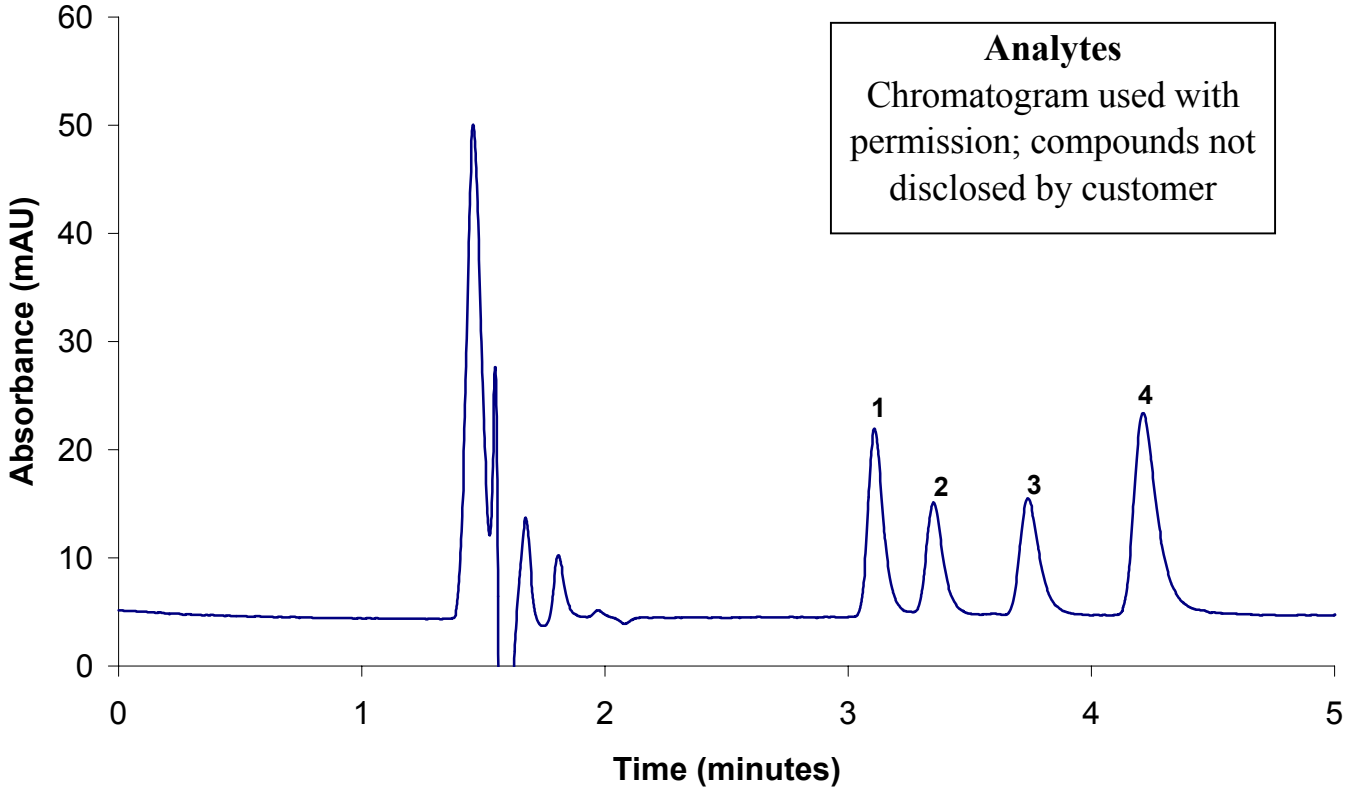
Flow rate:	1.0 mL/min.
Temperature:	40 ºC
Injection volume:	1 µL
Detection:	240 nm

ZirChrom®

Technical Bulletin #251

... For Peak Performance

Separation of Progestogen Steroids



LC Conditions

Column: DIAMOND BOND™ C18, 100 × 4.6 mm
Mobile Phase: 37.5/27.5/35 A/B/C
A: ACN
B: THF
C: Water

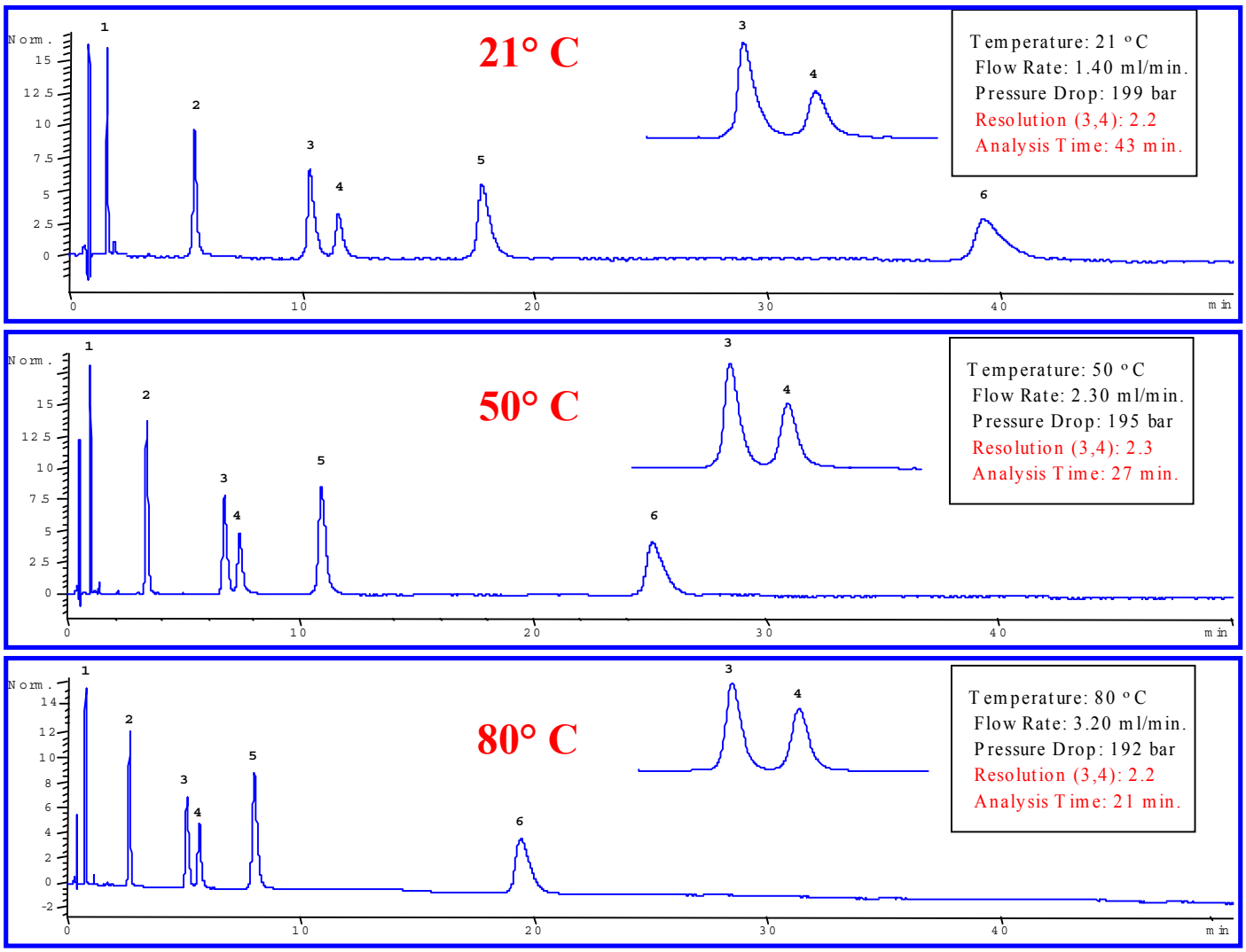
Flow rate:	1.0 mL/min.
Temperature:	75 ºC
Injection volume:	5 µL
Detection:	254 nm

ZirChrom®

Technical Bulletin #260

... For Peak Performance

Ultra Fast High Temperature HPLC on Diamondbond®-C18



Column: Diamondbond®-C18 100mm x 4.6mm

Solutes: 1-Barbital, 2-Butabarbital, 3-Pentobarbital, 4-Carbromal, 5-Secobarbital, 6-Methohexital

Mobile Phase: 21° C - Isocratic 18.5/81.5 A/B
50° C - Isocratic 13.5/86.5 A/B
80° C - Isocratic 8.80/91.2 A/B

A: Acetonitrile

B: 5mM Ammonium phosphate, pH 7.0

Temperature: See individual runs

Injection volume: 1.3 µL

Detection: 254 nm

Robust Methods with High Efficiency, Bonded-Carbon HPLC Columns

Technical Bulletin #268

Elena Khmelnitskaia
Cabot Corporation

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

Loss of stationary phase, retention drift, and short column life are common problems using bonded-phase silicas, especially with aggressive mobile phases. A new bonding technology overcomes these problems by attaching C18 groups to a carbon surface with ultra-stable carbon-carbon bonds. This note shows that robust methods with high efficiency are now possible using ultra-stable bonded-carbon columns.

Introduction

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

Bonded-Carbons

Zichrom Separations, Inc. and Cabot Corporation have developed new materials using unique technology to bond functional groups directly to the surface of carbon. 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 200 ^oC), and have not observed loss of bonded ligands.

Experimental

A method reliability test was set-up using 480 injections of a barbiturate mixture. A single DiamondBond™-C18 column (4.6 mm x 100 mm) was used for all of the injections. A new mixture of analytes was prepared after each 100 injections (analytes were purchased separately from Alltech). New mobile phase (10/15/75 THF/ACN/20 mM Ammonium Phosphate, pH 7.0) was also prepared fresh after each 100 injections.

Results

The results show that the separation has excellent long-term stability. Table 1 shows the average retention times for the analytes and the standard deviations. The relative standard deviations are generally 2% or lower.

Table 1 – Reproducibility of Barbiturate Method

Analyte	Avg. k'	St. Dev.	Relative St. Dev.
Barbital	0.46	0.011	2.4%
Metharbital	1.12	0.021	1.9%
Butethal	2.54	0.024	0.9%
Hexobarbital	3.18	0.044	1.4%
Mephobarbital	4.27	0.029	0.7%
Pentobarbital	4.92	0.063	1.3%

Figure 1 shows chromatograms for the first, 100th, 400th, and final injection. Note that this improvement in ligand stability also helps with LC/MS separations, since there is no ligand bleed to create noise in the MS baseline. ZirChrom's technical support group has extensive experience in this area, and would be happy to help you with your particular application.

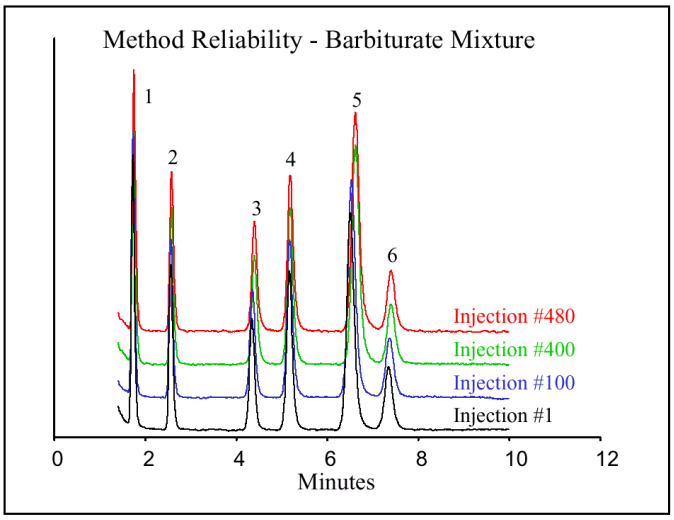


Figure 1: Barbiturate Method Reliability
1=Barbital, 2=Metharbital, 3=Butethal, 4=Hexobarbital, 5=Mephobarbital, 6=Pentobarbital

References

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

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Fast Separation of Nitrosamines

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

Technical Bulletin #279

Interest in the analysis of nitrosamine compounds is increasing as researchers become more aware of their toxicity and presence in the environment, foods, and pharmaceuticals. The extraordinary chemical and thermal stability of zirconia-based stationary phases allows for the rapid separation of these polar, basic compounds with good peak shape and efficiency. This note shows the separation of nine low molecular weight nitrosamines using a DiamondBond™-C18 column.

Introduction

N-nitrosamines are present in both food and the environment, and have been shown to be highly carcinogenic, with toxic levels as low as micrograms per kilogram. The nitrites and nitrates commonly used as preservatives in food can cause the reaction of secondary amines to form N-nitrosamines (1). Detection of these N-nitrosamines has been demonstrated using both UV detection at 230 nm (2) and mass spectrometry (3) coupled to liquid chromatography, however these separations are typically quite lengthy, ranging from 15-35 minutes.

The excellent chromatographic selectivity and thermal stability of zirconia-based phases allows much faster separation of these compounds at either high or low pH, where the electrospray ionization of the positively charged amines for detection by mass spectrometry is facilitated.

Experimental

A standard mixture of N-nitrosamines obtained from Supelco contained the following nine compounds: dimethylnitrosamine, ethylmethylnitrosamine, diethylnitrosamine, dipropylnitrosamine, dibutylnitrosamine, diphenylnitrosamine, nitrosomorpholine, nitrosopiperidine, nitrosopyrrolidine. The mixture was separated at 75 °C using a DiamondBond-C18 column using the following chromatographic conditions:

Column:	4.6 mm x 100 mm DiamondBond™-C18
Mobile Phase:	2.5-90% B from 1-3 minutes
	A = 10mM Ammonium hydroxide, pH 9.5
	B = ACN
Flow rate:	4.0 ml/min.
Injection Vol.:	1.0 µl
Detection:	UV at 230 nm

Even at a modest temperature of 75 °C the separation is rather fast, with full resolution of all nine compounds in just over 3 minutes.

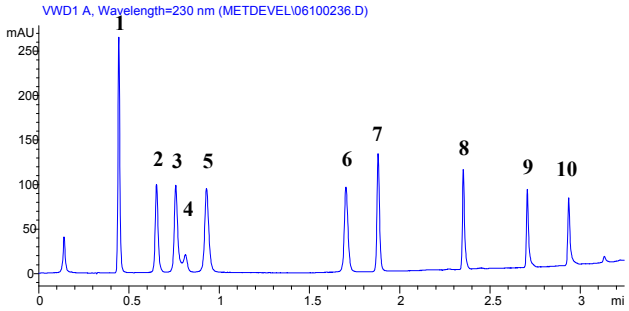

Figure 1: Separation of nitrosamines at 75 °C.

We note that our analysis shows ten peaks for the nine compound mixture. We believe peak number four in this separation may be an impurity in the mixture.

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 high efficiency with improved stability for extraordinary separations.

The authors thank Dr. Mourad Rahi of Pace Analytical for the Nitrosamine sample and helpful discussion.

References

(1) L. Cardenes et al. *J Chrom.,. A*, 2002; Vol. 946, pp 133-140.

(2) G. Bellec et al. *J. Chrom,. A*, 1996; Vol. 727, pp 83-92.

(3) D. Volmer et al. *Rapid Comm. In Mass Spec*., 1996; Vol, 10, pp 715-720.

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Fast Separation of Androsterone Steroids on DIAMONDBOND®-C18

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

Technical Bulletin # 284

This application note shows the separation of four closely related anabolic steroids (androsterone, epiandrosterone, etiocholanolone and epietiocholanolone) using a DiamondBond®-C18 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 (1). Baseline resolution of all four compounds was obtained on DiamondBond®-C18 at slightly elevated column temperature in under 3 minutes using isocratic elution.

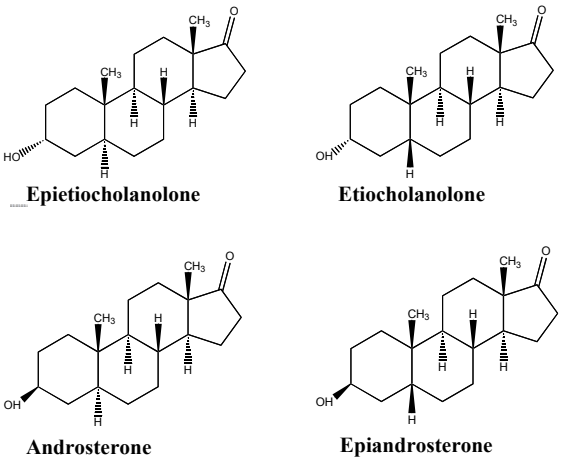

Figure 1: Structures of androsterone steroids.

Introduction

The rapid and accurate detection of anabolic steroids is crucial in today's sporting world. Historically the structural 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 due to their size and structure similarities and their nearly identical mass spectra.

Experimental

A mixture of androsterone steroids (see Figure 1) was separated at 100 ºC using a Diamondbond®-C18 column and a Metalox™ 200-C column heater. The separation conditions were as follows:

Column:	Diamondbond®-C18, 150 mm x 4.6 mm i.d. (Part Number: DB01-1546)
Mobile Phase:	60/40 acetonitrile/water
Temperature:	100 ºC with Metalox™ 200-C column heater
Flow Rate:	2 ml/min.
Injection Vol.:	10 µl
Pressure Drop:	148 bar
Detection:	UV at 215 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.

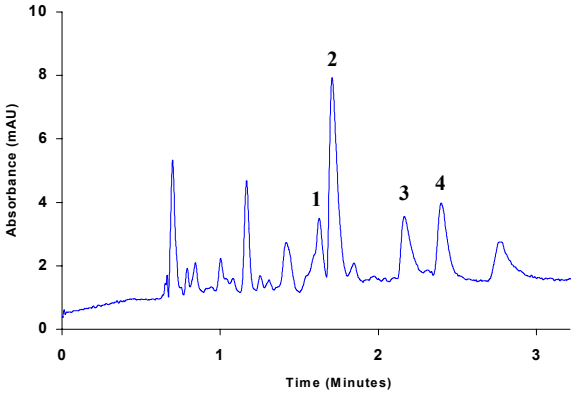

Figure 2: Separation of 1=Epietiocholanolone, 2=Etiocholanolone, 3=Androsterone, 4=Epiandrosterone on Diamondbond®-C18 at 100 ºC with the Metalox™ 200-C column heater.

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

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

References

(1) A. Leinonen et al., J. Mass Spectrometry; 37, 693-698 (2002).

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LC/MS/MS Method for Quantifying N-Nitrosamines on DIAMONDBOND®-C18

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

Technical Bulletin # 295

This application note shows the separation of nine pharmaceutical packaging related N-nitrosamines using a DiamondBond®-C18 column. Detection is by a mass spectrometer using Multiple Reaction Monitoring (MRM) mode of the characteristic MRM transition of each individual compound. The nine N-nitrosamines are quantitated by comparison to a standard curve. The method is suitable for the analysis of N-nitrosamine extractable/leachable at sub-ppb level for pharmaceutical containers.

Introduction

PPD Development, Inc. (http://www.ppdi.com) provides complete bioanalytical and GMP services for drug development. Bioanalytical laboratories are located in Madison, WI and Richmond, VA. A GMP laboratory is located in Madison, WI. PPD method development experts came to ZirChrom looking for assistance in developing an approach to quantify nine structurally similar N-nitrosamines in pharmaceutical packaging. Collaborative efforts led to the development and validation of the following LC/MS/MS method.

Table 1: Method Detection/Quantification Limits.
ng/mL (ppb) in water

Compound	LOD	LOQ
N-Nitrosodimethylamine (NDMA)	0.3	1.0
N-Nitrosodiethylamine (NDEA)	0.2	0.6
N-Nitrosomethylethylamine (NMEA)	0.04	0.12
N-Nitrosodi-n-propylamine (NDPA)	0.1	0.3
N-Nitrosodi-n-butylamine (NDBA)	0.04	0.12
N-Nitrosodiphenylamine (NDFA)	0.3	1.0
N-Nitrosomorpholine (NMOR)	0.2	0.6
N-Nitrosopiperidine (NPIP)	0.2	0.6
N-Nitrosopyrrolidine (NPYR)	0.2	0.6

Experimental

A mixture of N-nitrosamines (see Table 1) was separated at 50 ºC using a DiamondBond®-C18 column and a Metalox™ 200-C column heater. The separation conditions were as follows:

Column: DiamondBond®-C18, 100 mm x 4.6 mm i.d., 5 micron (Part Number: DB01-1046-5)

Mobile Phase: Gradient elution

Time	% A	% B
0	97.5	2.5
10	10	90
15	10	90
15.1	97.5	2.5
25	97.5	2.5

A: 0.1% (v/v) formic acid
B: acetonitrile

Temperature: 50 ºC with Metalox™ 200-C column heater
Flow Rate: 0.5 ml/min.
Injection: 0.1 ng
Detection: LC/MS/MS

These chromatographic conditions capitalize on the unique temperature stability and surface chemistry of zirconia-based stationary phases to achieve baseline resolution of these compounds in less than 13 minutes.

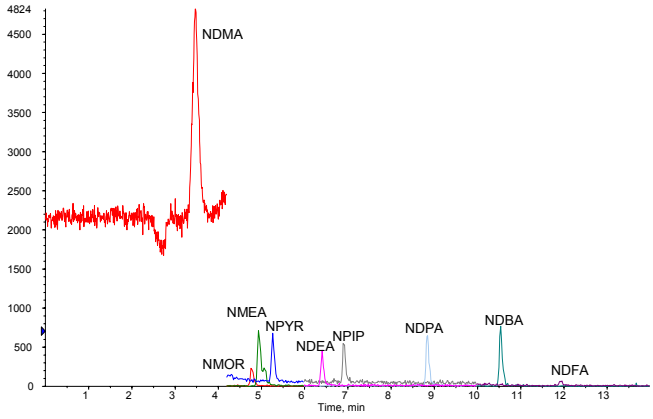

Figure 1: MRM chromatograms of nine N-nitrosamines at 0.1 ng injection.

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

T. J. Deng, Prasanna Sunthankar and Aryo Nikopour,
PPD Development, Inc., (Madison, Wisconsin, USA)

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