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.

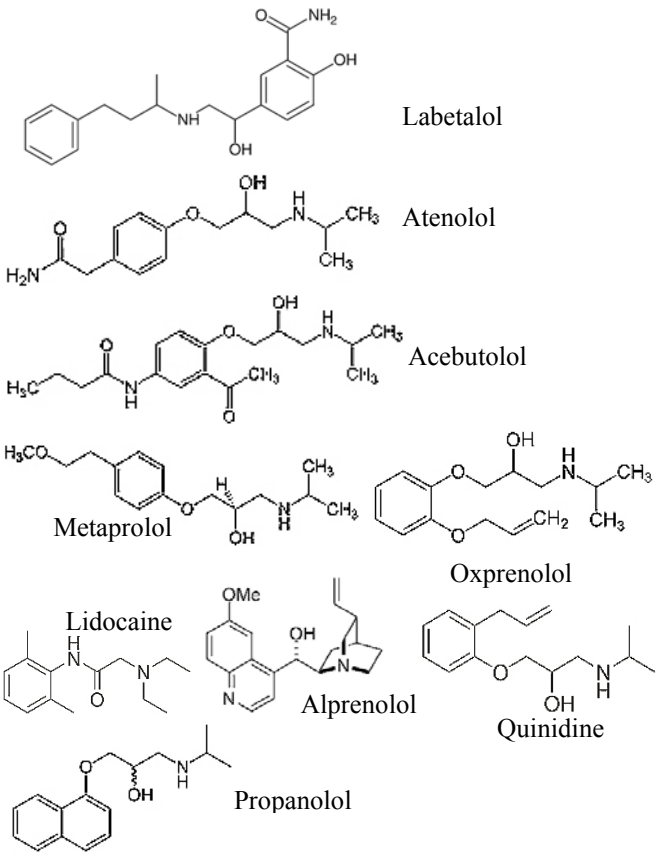

Figure 1. Structures of Pharmaceutical Compounds in Analysis

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

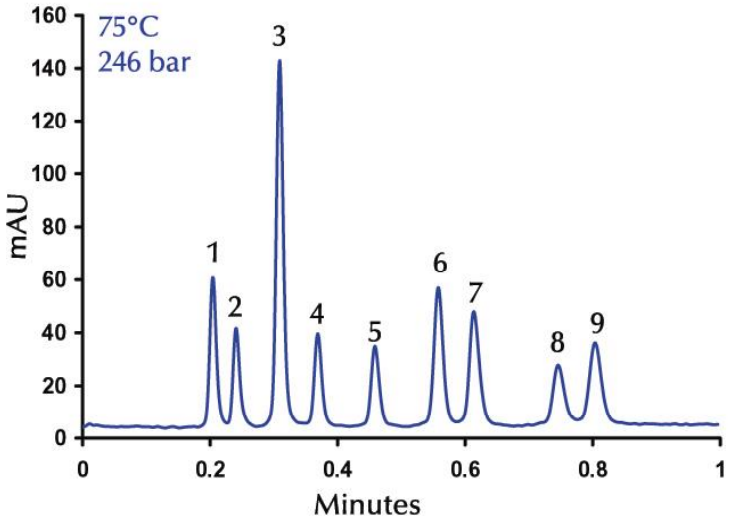

Figure 2. Separation of Nine Pharmaceuticals. **1**=Labetalol, **2**=Atenolol, **3**=Acebutolol, **4**=Metoprolol, **5**=Oxprenolol, **6**=Lidocaine, **7**=Quinidine, **8**=Alprenolol, **9**=Propranolol

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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ZirChrom®-MS Exhibits Unique Selectivity for Basic Pharmaceuticals

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

Technical Bulletin #299

Basic pharmaceuticals are well-known problematic compounds on silica C18 due to the interactions between the amine functionalities and non-bonded residual silanol groups[1]. In this application note we demonstrate the utility of a new Lewis acid deactivated zirconia-based column, ZirChrom®-MS. The new ZirChrom®-MS column exhibits unique selectivity for the specific set of amine-containing compounds studied.

Introduction

The chromatography of basic pharmaceuticals on silica C18 has traditionally been so problematic that amitriptyline is commonly used as a probe solute for quantifying silanophilicity of silica phases. The surface chemistry of zirconia-based phases is dominated by Lewis acid sites, rather than the Bronsted acid sites, which dominate the surface chemistry of silica phases. The mixed-mode retention character of ZirChrom®-MS (cation-exchange and reversed-phase) allows separations that were previously difficult using conventional silica C18 phases.

Using traditional, near normal pH operating conditions one typically obtains significantly higher retention for basic compounds on ZirChrom®-MS versus traditional silica C18. Even though there is much higher carbon loading to silica-based columns the strong ion-exchange contribution to retention results in an overall higher retention factor on the ZirChrom®-MS column. In fact, the ZirChrom®-MS phase has relatively higher retention for basic drugs compared to all of the zirconia-based reversed phases as well. In general, excellent peak shapes may be obtained using LC/MS compatible, near neutral pH operating conditions.

In addition, ZirChrom®-MS enables the user to analyze basic pharmaceutical compounds, acidic pharmaceutical compounds, or both simultaneously, under LC/MS compatible, near neutral pH operating conditions.

Experimental

The selectivity of a set of basic pharmaceuticals was compared using a leading silica C18 column and a ZirChrom®-MS column. The separation conditions were as follows:

Column Size:	50 mm x 4.6 mm i.d.
Mobile Phase:	Isocratic elution: 65/35 A/B A: methanol B: 25mM ammonium phosphate, pH 6.0
Temperature:	35 ºC
Flow Rate:	1.0 ml/min.
Injection Vol.:	5 µl
Detection:	UV at 254 nm
Solutes:	(From left to right in **Figure 1**) Methapyrilene, Pyrilamine, Tripelennamine, Brompheniramine, Desipramine, Nortryptyline, Doxepin, Amitryptyline

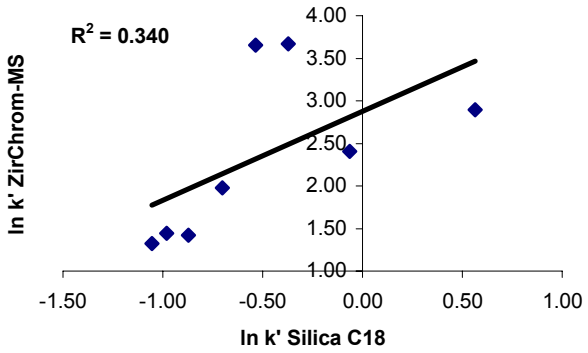

Figure 1: Selectivity Comparison for a Set of Basic Pharmaceuticals - leading Silica C18 versus ZirChrom®-MS.

As a result of the mixed-mode ion-exchange and reversed-phase characteristics of ZirChrom®-MS, the elution order of basic pharmaceuticals is often quite different compared to leading reversed-phase silica phases. **Figure 1** shows a plot of ln k' for eight common basic pharmaceuticals on a leading silica C18 phase versus ln k' for the same compounds on ZirChrom®-MS. There is no apparent correlation of the retention for these compounds on the silica C18 phase with the retention on ZirChrom®-MS. This different selectivity is particularly useful in method development for basic pharmaceuticals. When a pair of basic compounds cannot be separated using a traditional silica C18 phase, the chances of them separating on ZirChrom®-MS are much better than on any other silica phase.

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

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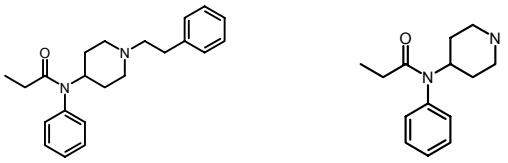
Simultaneous Extraction and Quantitation of Fentanyl and Norfentanyl from Primate Plasma with LC/MS Detection

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)

References

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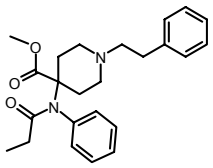
Extraction and Quantitation of Carfentanil and Naltrexone in Mammalian Plasma with LC/MS Detection

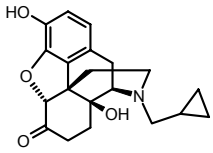
Clayton McNeff, Ph.D. and Steven Rupp
ZirChrom Separations, Inc.

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

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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.

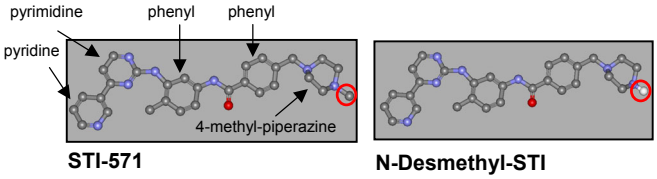

Figure 1. Structures of STI-571 and N-Desmethyl-STI

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)

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The Analysis of Basic Compounds Using Neutral pH Conditions: A Column Comparison Study

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

Technical Bulletin # 303

ZirChrom®-MS represents another first of its kind zirconia-column designed specifically for MS detection and the high demands of pharmaceutical method development specifications. Using our novel covalently attached Lewis acid deactivation chemistry, ZirChrom has developed a highly retentive reversed-phase HPLC column, which is easy to use and which still has the inherent chemical stability advantages of zirconia-based HPLC columns. Most importantly this new column still maintains the very different chromatographic selectivity, especially for basic pharmaceuticals that zirconia-based columns are well known to have compared to traditional bonded C18 silica phases. This new column compliments the family of reversed phase columns that ZirChrom currently markets; a family of chemically different and thermally stable HPLC columns.

Introduction

ZirChrom®-MS is a surface deactivated, reversed-phase zirconia column designed specifically for LC-MS applications, particularly those involving basic pharmaceutical compounds. The following unique features make ZirChrom®-MS an ideal choice for today's LC-MS method developer:

1. Compatible with volatile, near neutral pH mobile phase buffers including ammonium acetate and formate.
2. Enhanced retention for basic pharmaceutical compounds compared to bonded phase C18 silica under LC-MS compatible operating conditions.
3. Very different chromatographic selectivity for basic drugs compared to bonded phase C18 silica using LC-MS conditions.
4. Improved peak shape and efficiency for basic drugs compared to bonded phase C18 silica using LC-MS conditions.
5. The ability to analyze basic, acidic or neutral pharmaceutical compounds, or mixtures of all three, simultaneously.
6. Low column bleed characteristics due to covalent bonding chemistry.

Experimental

A column comparison study using pharmaceutically relevant compounds was performed to demonstrate the unique characteristics and excellent performance of ZirChrom®-MS relative to a leading bonded phase C18 silica column. The separation conditions were as follows:

Columns:	ZirChrom®-MS (Part Number: MS01-0546) 50 mm x 4.6 mm i.d., 3µm particle size; Leading bonded phase C18 silica, 150 mm x 4.6 mm i.d., 3.5 µm particle size
Mobile Phase:	Machine-mixed 80/20 ACN/10 mM ammonium acetate, pH=6.7 without pH adjustment
Temperature:	35 ºC
Flow Rate:	1.0 ml/min.
Injection:	0.1 µl
Detection:	UV at 254 nm

The following 26 compounds were included in the test set:

(1) Methapyrilene, (2) Pyrilamine, (3) Tripeleneamine,
(4) Chlorpheniramine, (5) Brompheniramine, (6) Thiothixene,
(7) Doxepin, (8) Amitryptyline, (9) Desipramine,
(10) Nortryptyline, (11) Pyridine, (12) Imipramine, (13) Lidocaine,
(14) Atenolol, (15) Metoprolol, (16) Oxprenolol, (17) Alprenolol,
(18) Phenol, (19) 4-chlorophenol, (20) Acetaminophen,
(21) Ketoprofen, (22) Ibuprofen, (23) Naproxen, (24) Toluene,
(25) Biphenyl, (26) Phenanthrene.

Compounds (1) thru (17) are basic; compounds (18) thru (23) are acidic; and compounds (24) thru (26) are neutral test probes.

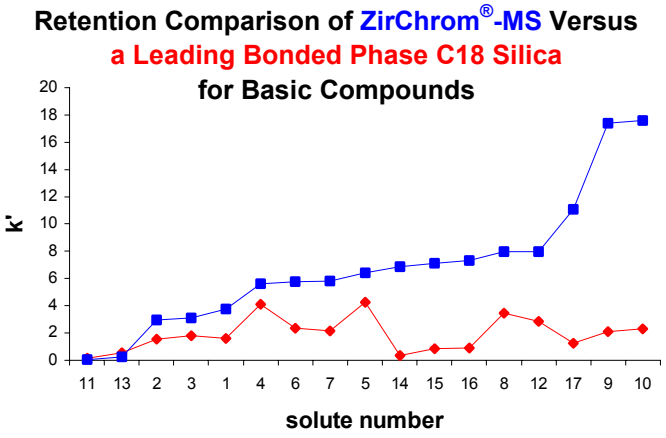

Figure 1. Retention Comparison for Basic Compounds.

Figure 1 shows a retention comparison of ZirChrom®-MS versus a leading bonded phase C18 silica for basic compounds under these LC-MS compatible operating conditions. For illustrative purposes the solutes are organized in order of increasing retention on ZirChrom®-MS. This figure demonstrates that ZirChrom®-MS offer enhanced retention for basic pharmaceutical compounds compared to bonded phase C18 silica.

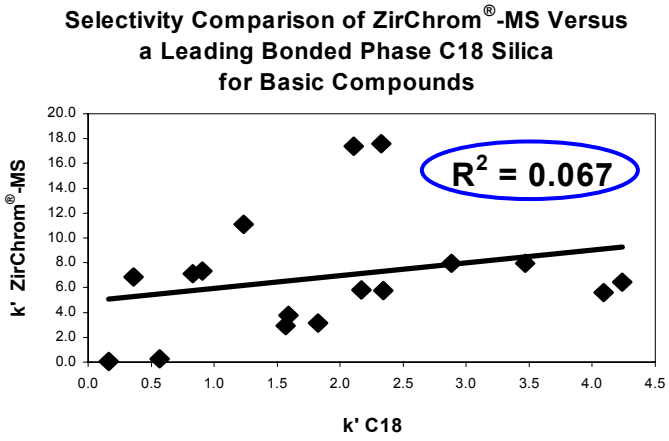

Figure 2. Selectivity Comparison for Basic Compounds.

Figure 2 shows a selectivity comparison of ZirChrom®-MS versus a leading bonded phase C18 silica for basic compounds under these LC-MS compatible operating conditions. This figure demonstrates that ZirChrom®-MS offers very different chromatographic selectivity (R^2 = 0.067) for basic drugs compared to bonded phase C18 silica.

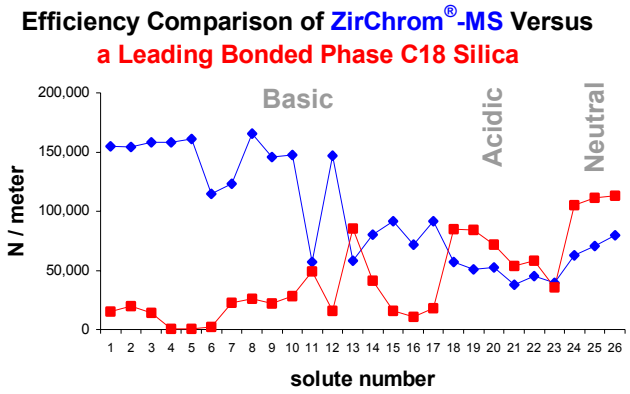

Figure 3. Efficiency Comparison for All Compounds.

Figure 3 shows the efficiency comparison of ZirChrom®-MS versus a leading bonded phase C18 silica for all compounds under these LC-MS compatible operating conditions. ZirChrom®-MS produced superior column efficiency (plates per meter) in 16 out of 17 cases involving basic compounds. The leading bonded phase C18 silica only produced acceptable column efficiency in the cases involving acidic and neutral compounds.

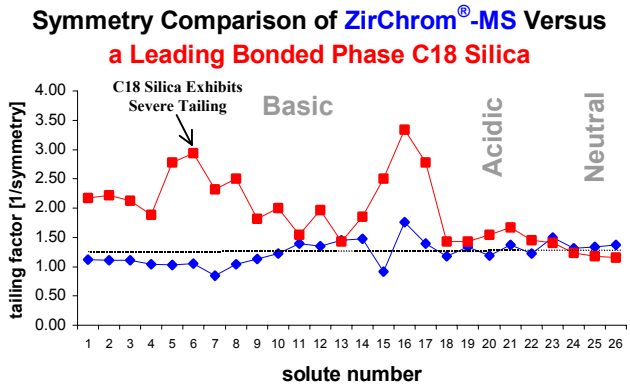

Figure 4. Symmetry Comparison for All Compounds.

(Note: tailing factor was calculated by the formula [1/symmetry] using the symmetry value as reported by the Agilent® 1100 Chemstation® software.)

Figure 4 shows the symmetry comparison of ZirChrom®-MS versus a leading bonded phase C18 silica for all compounds under these LC-MS compatible operating conditions. ZirChrom®-MS produced superior column symmetry in 16 out of 17 cases involving basic compounds. The leading bonded phase C18 silica only produced acceptable column symmetry in the cases involving acidic and neutral compounds.

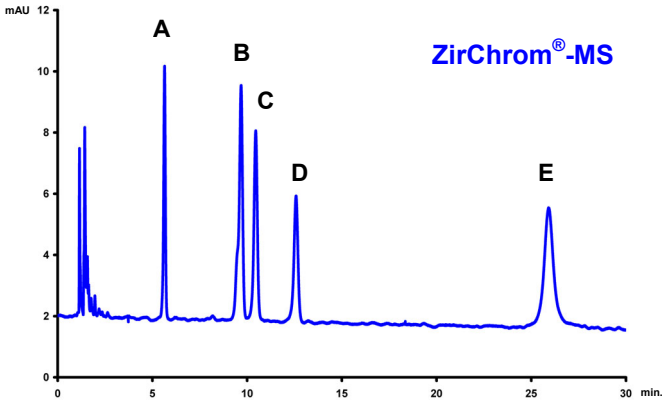

Figure 5. ZirChrom®-MS Separation of Basic Compounds.
Elution Order: (A) Methapyrilene, (B) Brompheniramine, (C) Doxepin, (D) Amitriptyline, (E) Nortryptyline.
Note: Column used was 150 mm x 4.6 mm i.d., 3µm particle size.

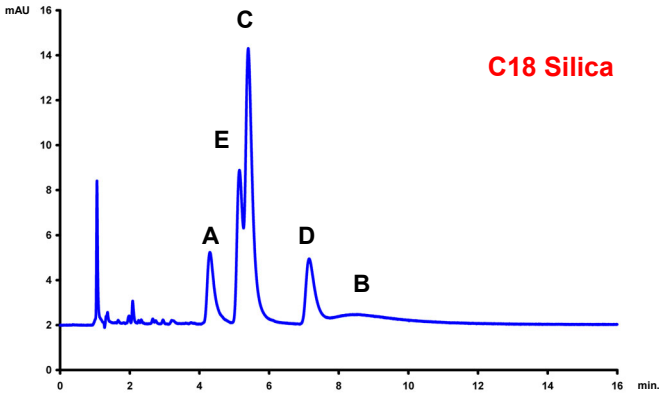

Figure 6. Leading Bonded Phase C18 Silica Separation.
*Note: The basic compounds are lettered the same as in **Figure 5.***

Figures 5 & 6 show a representative separation involving some of the basic compounds used in the study. Clearly, ZirChrom®-MS offers both unique selectivity and superior chromatographic performance relative to a leading bonded phase C18 silica for basic compounds under these LC-MS compatible operating conditions.

Summary

In summary, ZirChrom®-MS consistently outperformed a leading bonded phase C18 silica for the separation of basic compounds under LC-MS compatible operating conditions. ZirChrom®-MS produced enhanced retention, unique selectivity, greater efficiency and improved symmetry for virtually all of the basic compounds that were studied.

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