LC/MS Compatible Separation of Antidepressants on ZirChrom®-MS

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

Technical Bulletin # 296

The chromatography of basic drugs on C18-silica phases is often complicated due to mixed-mode interactions that cause poor peak shape and irreproducible results. ZirChrom®-MS is a new zirconia-based reversed-phase column that has enhanced mixed mode retention characteristics which allow for LC/MS chromatography of highly basic amines with excellent peak shape and efficiency.

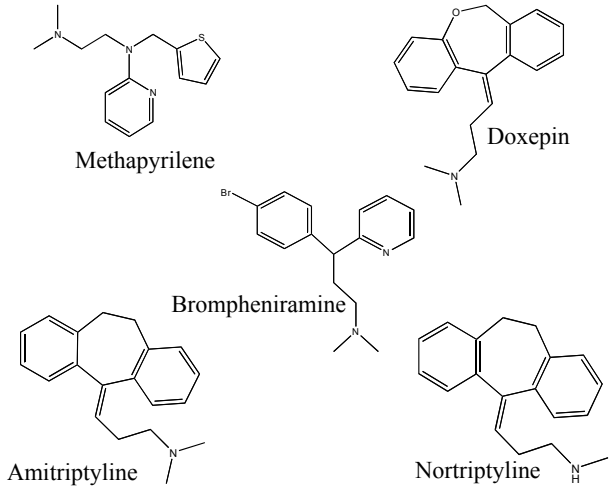

Figure 1: Structures for the Compounds of Interest.

Introduction

The chromatography of basic drugs on C18-silica phases is difficult due to interactions between silanols and the amines[1]. We have found that a tricyclic antidepressant, Amitriptyline, may be used as a probe solute for quantifying silanophilicity of HPLC columns. 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. This application note shows an impressive LC/MS compatible separation of antidepressants in a highly organic, near neutral pH mobile phase.

Experimental

A mixture of five basic drugs (four antidepressants) was separated at 35 ºC using a ZirChrom®-MS column. The separation conditions were as follows:

Column:	ZirChrom®-MS, 50 mm x 4.6 mm i.d. (Part Number: MS01-0546)
Mobile Phase:	Isocratic elution: 65/35 A/B A: acetonitrile B: 10mM ammonium acetate, pH 5.0
Temperature:	35 ºC
Flow Rate:	1.0 ml/min.
Injection Vol.:	1 µl
Pressure Drop:	59 bar
Detection:	UV at 254 nm

Five basic pharmaceutical compounds were separated using simple acetonitrile/water isocratic elution and a LC/MS friendly acetate buffer. The selectivity and peak symmetry of all five compounds is very good which allows for a separation using only a short 5 cm long column.

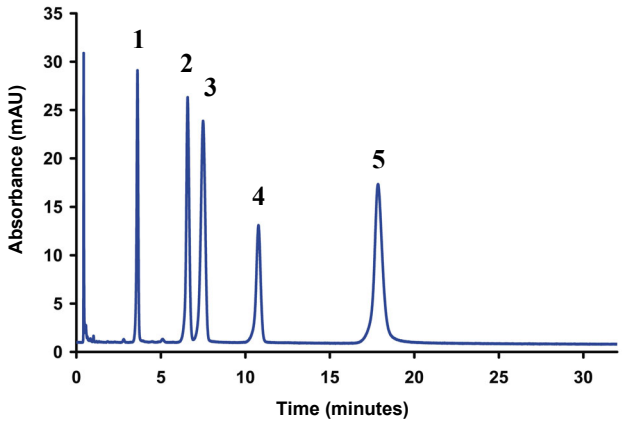

Figure 2: Separation of 1=Methapyrilene, 2=Doxepin, 3=Brompheniramine, 4=Amitriptyline, and 5=Nortriptyline.

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) G.B. Cox, *J. Chromatography A*. **656**, 353, 1993.

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LC/MS Compatible Separation of Non-Steroidal Anti-Inflammatory Drugs on ZirChrom®-MS

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

Technical Bulletin #297

The Lewis acidity of zirconia-based supports for HPLC has historically presented problems in the analysis of analytes containing Lewis base moieties, such as carboxylates, particularly in LC/MS applications where volatile mobile phase additives are required. In this application note we demonstrate the utility of a new Lewis acid deactivated zirconia-based column, ZirChrom®-MS.

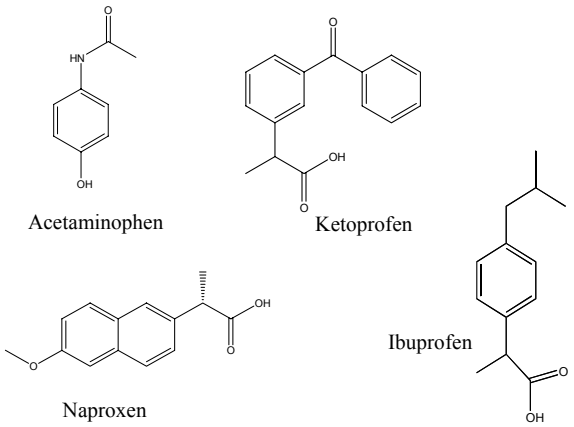

Figure 1: Structures of Non-steroidal anti-inflammatory drugs.

Introduction

Historically, the Lewis base carboxylic acid moiety on non-steroidal anti-inflammatory drugs required the use of a Lewis base mobile phase additive of a higher strength in the elutropic series (such as phosphate or fluoride) (1). While these types of additives work well in applications with UV/Vis detection, their use is almost entirely prohibited in LC/MS applications due to their relatively low volatility.

The deactivation of Lewis acid sites on the surface of the ZirChrom®-MS particle allows the chromatography of Lewis base analytes using mobile phase additives of the users choice including conventional LC/MS compatible buffers (such as acetate and formate) throughout the pH range of 1-10.

Experimental

Four non-steroidal anti-inflammatory drugs were separated at 35ºC using a ZirChrom®-MS column. The separation conditions were as follows:

Column:	ZirChrom®-MS, 50 mm x 4.6 mm i.d. (Part Number: MS01-0546)
Mobile Phase:	Isocratic elution: 40/60 A/B A: acetonitrile B: 10mM ammonium acetate, pH 5.0
Temperature:	35 ºC
Flow Rate:	1.0 ml/min.
Injection Vol.:	5 µl
Pressure Drop:	68 bar
Detection:	UV at 254 nm

Four non-steroidal anti-inflammatory drugs were separated using simple acetonitrile/water isocratic elution and a LC/MS friendly acetate buffer. The selectivity of all four compounds is excellent which allows for a very good separation using only a short 5 cm column.

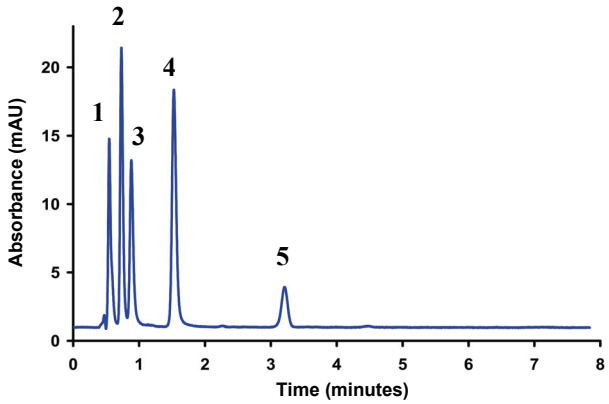

Figure 2: Separation of 1=Acetaminophen, 2=Ketoprofen, 3=Naproxen, 4=Ibuprofen, and 5=Impurity.

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.

References

(1) Blackwell, J. A.; Carr, P. W., *Journal of Liquid Chromatography*, *14*, 2875-2889, **1991**.

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LC/MS Compatible Separation of Beta-Blockers on ZirChrom®-MS

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ZirChrom Separations, Inc.

Technical Bulletin # 298

The chromatography of basic pharmaceuticals (with amine functionalities) on C18-silica phases can be difficult due to secondary interactions that cause tailed peaks in the neutral pH range where most silica phases are stable[1]. ZirChrom®-MS is a new zirconia-based reversed-phase column that has mixed mode retention characteristics which allow for LC/MS chromatography of highly basic amines with excellent peak shape and efficiency.

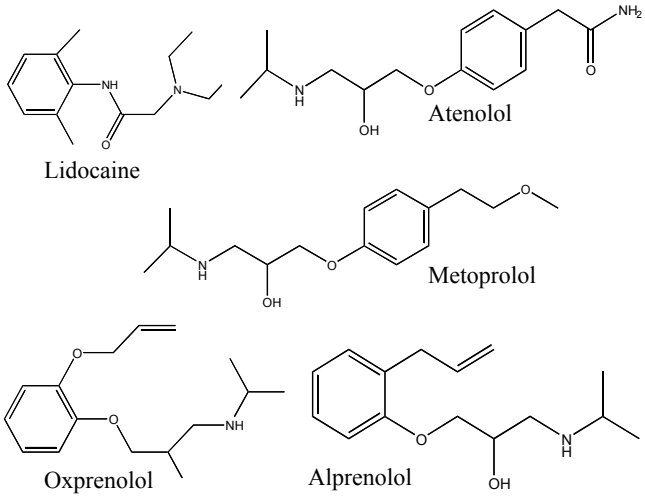

Figure 1: Structures for the Compounds of Interest.

Introduction

The chromatography of basic drugs on C18-silica phases has traditionally been problematic. 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. This application note shows an impressive LC/MS compatible separation of beta-blockers in a highly organic, near neutral pH mobile phase.

Experimental

A mixture of five amine-containing compounds was separated using a ZirChrom®-MS column at 35 ºC. The separation conditions were as follows:

Column:	ZirChrom®-MS, 50 mm x 4.6 mm i.d. (Part Number: MS01-0546)
Mobile Phase:	Isocratic elution: 65/35 A/B A: acetonitrile B: 10mM ammonium acetate, pH 5.0
Temperature:	35 ºC
Flow Rate:	1.0 ml/min.
Injection Vol.:	5 µl
Pressure Drop:	59 bar
Detection:	UV at 254 nm

Five Beta-Blockers were separated using simple acetonitrile/water isocratic elution and an LC/MS friendly acetate buffer. The selectivity of all five compounds is excellent using only a short 5 cm long column.

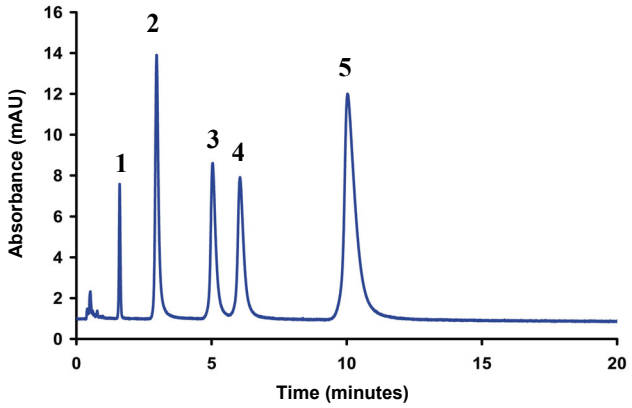

Figure 2: Separation of 1= Lidocaine, 2= Atenolol, 3= Metoprolol, 4= Oxprenolol, and 5= Alprenolol.

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) G.B. Cox, *J. Chromatography A*. **656**, 353, 1993.

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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

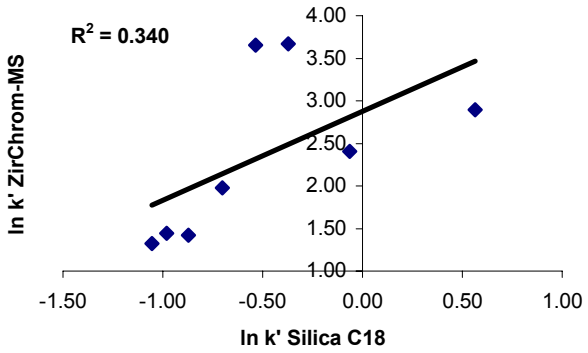

Figure 1: Selectivity Comparison for a Set of Basic Pharmaceuticals - leading Silica C18 versus ZirChrom®-MS.

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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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.

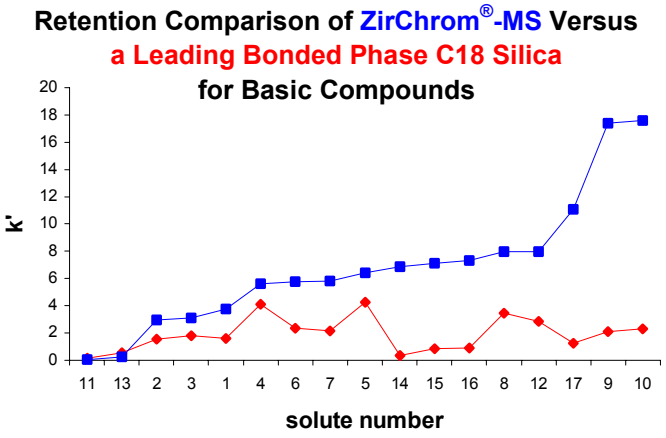

Figure 1. Retention Comparison for Basic Compounds.

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.

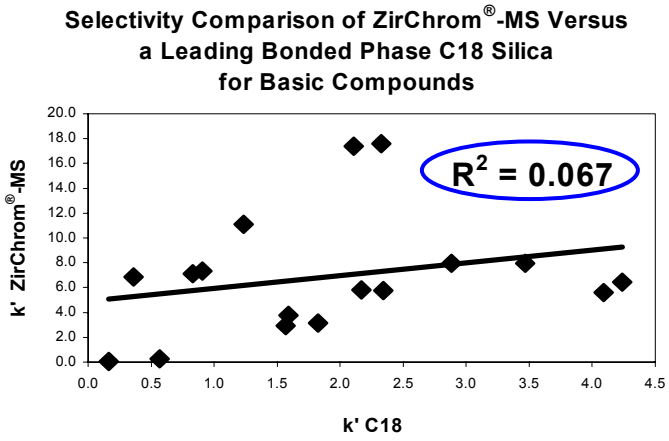

Figure 2. Selectivity Comparison for Basic Compounds.

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.

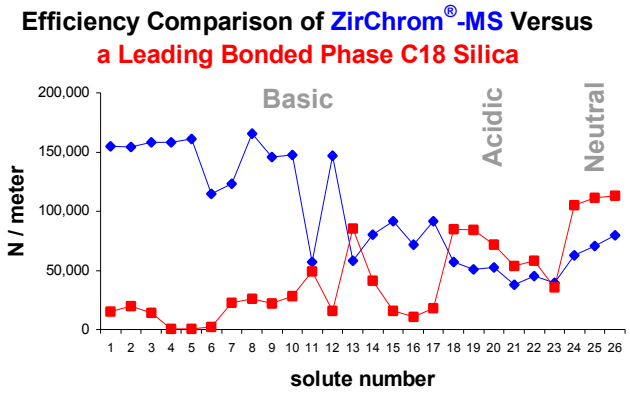

Figure 3. Efficiency Comparison for All Compounds.

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.

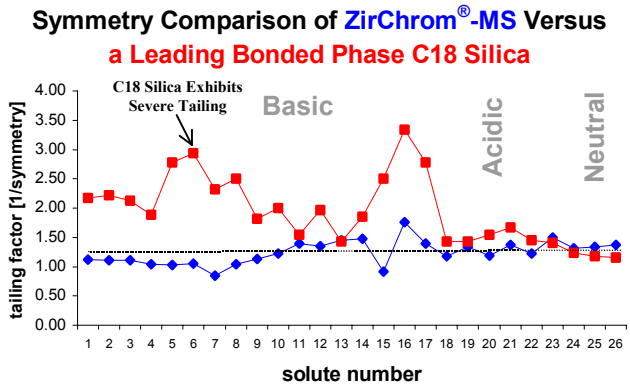

Figure 4. Symmetry Comparison for All Compounds.

(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.

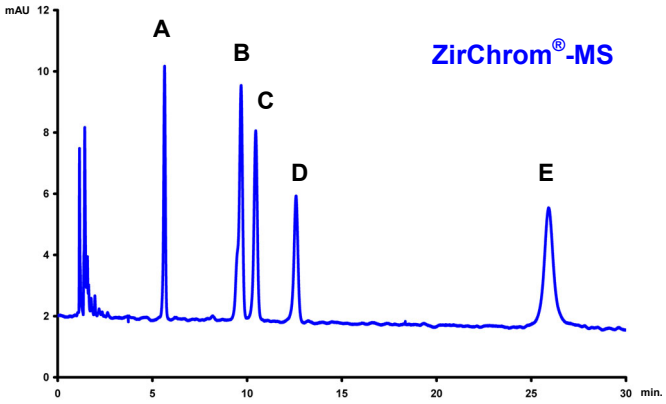

Figure 5. ZirChrom®-MS Separation of Basic Compounds.
Elution Order: (A) Methapyrilene, (B) Brompheniramine, (C) Doxepin, (D) Amitriptyline, (E) Nortryptyline.
Note: Column used was 150 mm x 4.6 mm i.d., 3µm particle size.

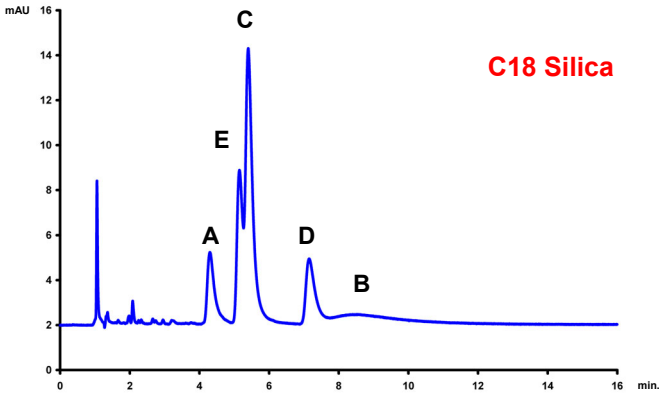

Figure 6. Leading Bonded Phase C18 Silica Separation.
*Note: The basic compounds are lettered the same as in **Figure 5.***

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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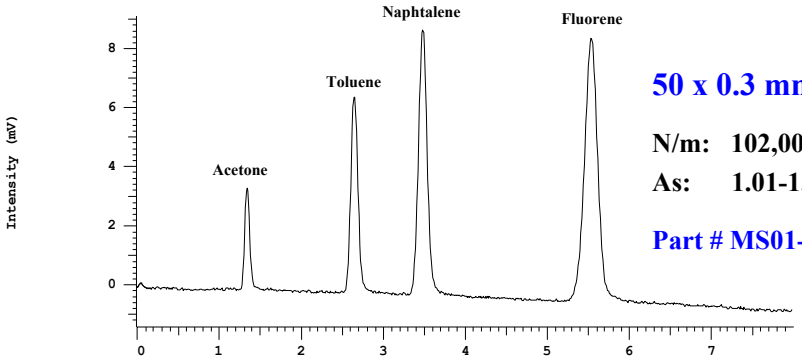
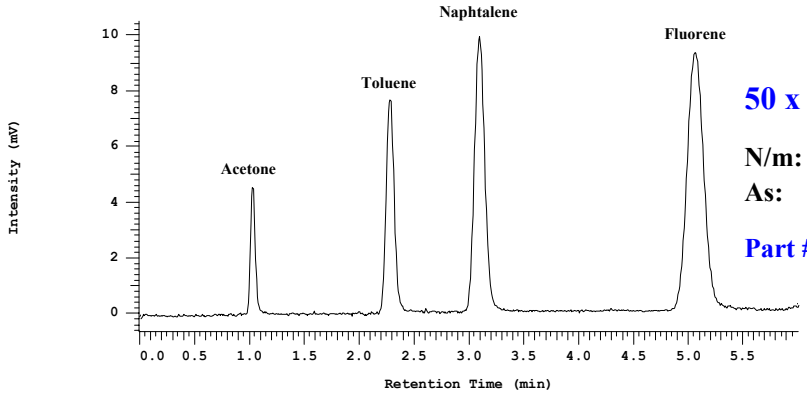


50 x 0.3 mm i.d.

N/m: 102,000-122,000
As: 1.01-1.06

Part # MS01-0503 (3 µm)



50 x 0.5 mm i.d.

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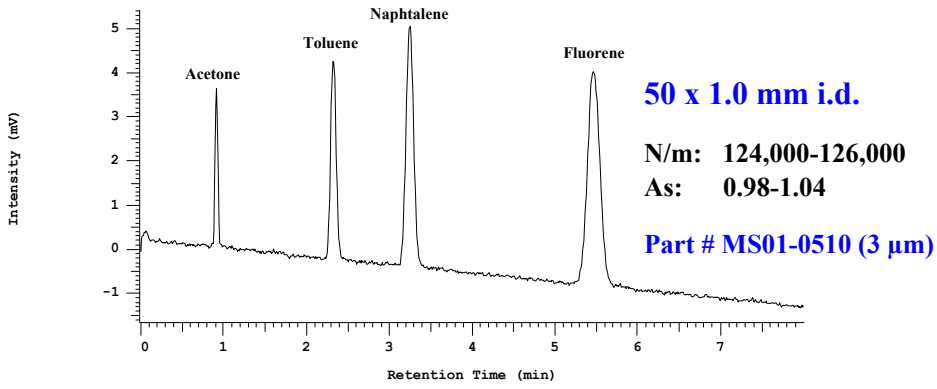

50 x 1.0 mm i.d.

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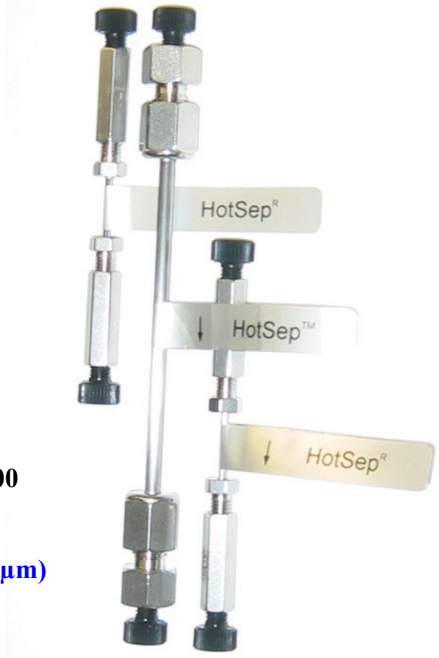

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(B) 0.5 mm ID, flow rate: 8 µL/min
(C) 1.0 mm ID, flow rate: 30 µL/min

Mobile phase: ACN – water (70:30, v/v). UV-detection at 254 nm.