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

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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.

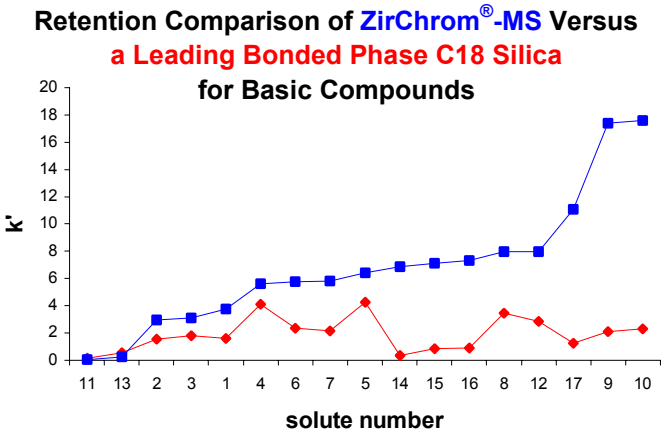

Figure 1. Retention Comparison for Basic Compounds.

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.

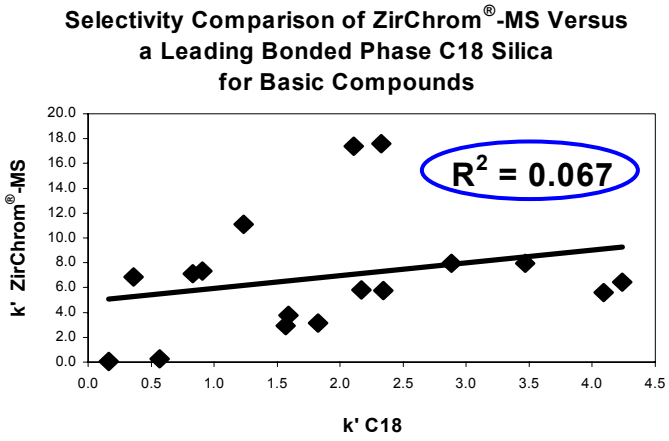

Figure 2. Selectivity Comparison for Basic Compounds.

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.

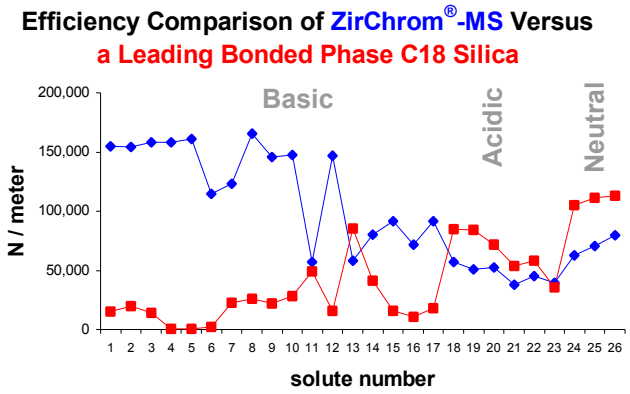

Figure 3. Efficiency Comparison for All Compounds.

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.

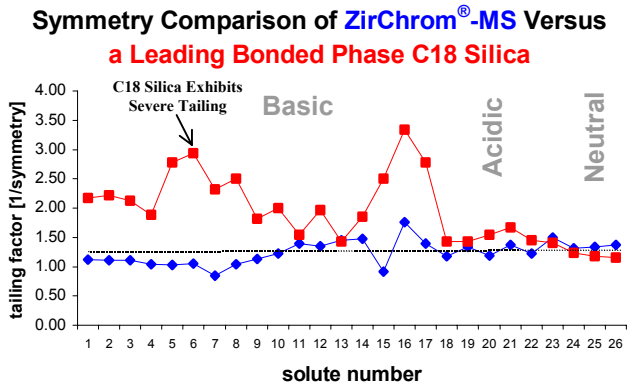

Figure 4. Symmetry Comparison for All Compounds.

(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.

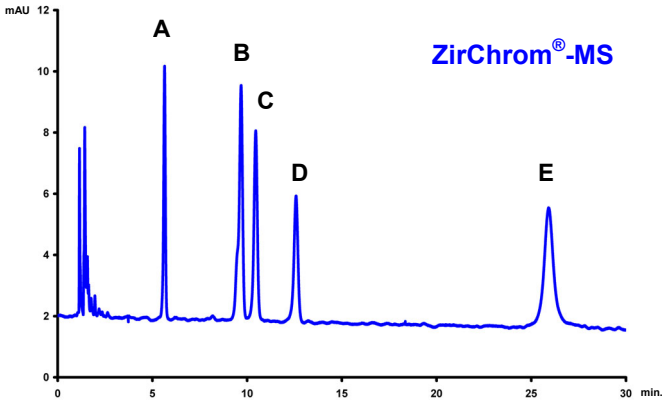

Figure 5. ZirChrom®-MS Separation of Basic Compounds.
Elution Order: (A) Methapyrilene, (B) Brompheniramine, (C) Doxepin, (D) Amitriptyline, (E) Nortryptyline.
Note: Column used was 150 mm x 4.6 mm i.d., 3µm particle size.

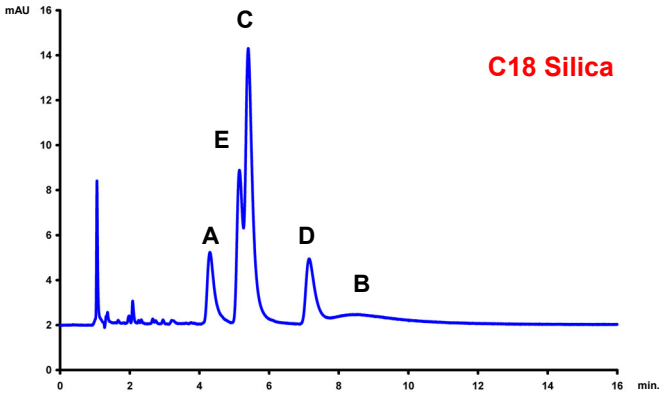

Figure 6. Leading Bonded Phase C18 Silica Separation.
*Note: The basic compounds are lettered the same as in **Figure 5.***

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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