ZirChrom®-EZ – The Next Generation Zirconia-Based RPLC Column

Welcome to the fourth issue of ZirChrom's electronic newsletter.

This newsletter issues approximately every 6 weeks and will address both general topics of interest to all chromatographers and specific features and benefits of ZirChrom's family of ultra-stable zirconia-based HPLC phases. All issues discuss practical topics to help you improve your productivity and understanding of chromatography.

HPLC continues to dominate other available analytical techniques for both quantitative and qualitative analysis of non-volatile substances, especially for pharmaceutical analyses. As this technique has matured, the number of available stationary phases seems to be increasing at an exponential rate. However, reversed-phase continues to be the preferred mode of HPLC for a broad range of analyses. The very large family of reversed phases that are commercially available retain a broad variety of analyte classes. The rate of introduction of new reversed-phase HPLC columns has increased rapidly over time. Indeed, nearly 80 new LC stationary phases were introduced in 1998/99 alone [1]. Several major directions in the development of new column technology are clear:

- Improved column stability
- Better column-to-column and batch-to-batch reproducibility
- Faster analysis
- Improved performance for cationic (basic) analytes
- Unique selectivities
- Columns designed for specific applications such as LC/MS

Many new phases have resulted from research directed at producing more stable materials [2, 3]. A number of studies have centered on understanding and minimizing silanol-analyte interactions and have worked to address the need for more selective separations in all modes of LC [4, 5]. Most of this new phase development work focuses on silica-based materials [1]. Silica's popularity is due to its long history in chromatography, its excellent separation efficiency, and the ease with which it can be modified to create new stationary phases. However, silica's inherent pH and thermal instability significantly limits the conditions under which silica-based columns can be used, especially under reversed-phase conditions.

New support materials specifically designed to overcome these shortcomings include synthetic organic polymers [6], alumina [7], and other metal oxides [8]. We at ZirChrom believe that porous zirconia comes closest to being an ideal liquid chromatographic support. The major advantage of zirconia over silica and alumina is its chemical stability. Derivatized silica-based stationary phases degrade outside the pH range 2 to 8 (at 35 °C) [9, 10] and alumina dissolves outside the pH range of 3 to 12 (at 35 °C). This enhanced chemical stability allows for the use of zirconia-based HPLC supports under a wide variety of conditions that rapidly destroy silica- and alumina-based columns.

However, in some cases reversed-phase HPLC method development is hampered with zirconia due to the inherent differences between the surface chemistries of zirconia and silica gel. The Lewis acid-base chemistry of zirconia has been extensively studied [11]. The

three types of chromatographically important sites on zirconia are a) Brönsted acid, b) Brönsted base and c) Lewis acid, which are shown in Figure 1. However, for all practical purposes, the Lewis acid sites typically dominate the surface chemistry of zirconia due to the ease with which these sites can adsorb constituents from the mobile phase to form a chemisorbed layer on the zirconia surface. The worst case scenario from the perspective of method development is that Lewis base moieties in analyte molecules can adsorb to these Lewis acid sites causing irreversible adsorption of the analyte. New research performed at ZirChrom has resulted in a solution to this complexity in using zirconia-based media with the introduction of ZirChrom®-EZ! The new ZirChrom®-EZ zirconia-based RPLC column is modified with a metal chelator group that effectively "deactivates" the Lewis acid sites, and is chemically stable from pH 1 to 10.

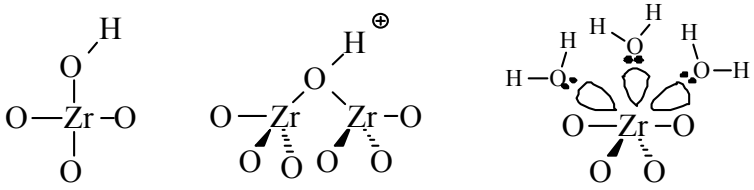

Figure 1. Schematic of major species present on the zirconia surface; A) Brönsted acid site, b) Brönsted base site, and c) Lewis acid site (ligand exchange).

Chromatographic Testing of ZirChrom®-EZ

In order to demonstrate the new capabilities of ZirChrom®-EZ, and compare it to our non-deactivated ZirChrom®-PBD phase, we selected some Lewis bases such as alkoxybenzoic acids, which interact strongly with the ZirChrom®-PBD column, causing broad and tailed peaks unless an additive like phosphate is added to the mobile phase. Figure 2 shows the separation of several Lewis base solutes (4-hydroxybenzoic acid, 4-ethoxybenzoic acid, 4-propoxybenzoic acid, and 4-butoxybenzoic acid) on ZirChrom®-EZ in a mobile phase containing only acetonitrile and water. This separation is obtained without any mobile phase additives, but with excellent peak shape and good efficiency. Now there is a choice when doing this analysis; ZirChrom®-PBD should be used when non-volatile buffers such as phosphate may be used, and ZirChrom®-EZ should be used for MS-detection that requires volatile buffers.

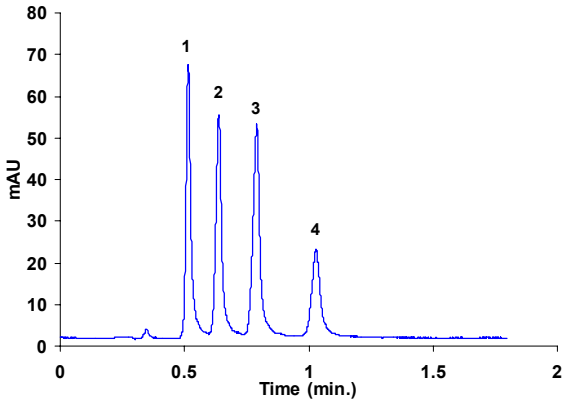

Figure 2. Separation of Lewis base solutes on ZirChrom®-EZ, 50 mm x 4.6 mm i.d. (P/N, EZ01-0546); (1) 4-hydroxybenzoic acid, (2) 4-ethoxybenzoic acid, (3) 4-propoxybenzoic acid, and (4) 4-butoxybenzoic acid. **LC Conditions**: Mobile phase, 40/60 ACN/Water; Flow rate, 1.0 ml/min; Temperature, 30 °C with Metalox™ 200C; Detection at 254 nm; Injection volume, 1 µl.

A good example where the Lewis acidity of zirconia-based supports for HPLC has historically presented problems is in the analysis of acidic pharmaceuticals such as non-steroidal anti-inflammatory drugs (NSAIDs), particularly in LC/MS applications where volatile mobile phase additives are required. While hard Lewis base type additives such as phosphate work well in applications with UV/Vis detection, their use is almost entirely prohibited in LC/MS applications due to their relatively low volatility. Here we demonstrate the utility of the new ZirChrom®-EZ column for the analysis of this class of compounds using a small amount (20 mM) of volatile buffer to assure adequate control of the mobile phase pH. Figure 3 shows the separation of five non-steroidal anti-inflammatory drugs, each containing a carboxylate functionality, using simple acetonitrile/water gradient elution and a LC/MS friendly acetate buffer. Excellent peak shape and efficiency is obtained for each component under these conditions. This is one example of how the new ZirChrom®-EZ reversed-phase is complementary to the ZirChrom®-PBD reversed-phase.

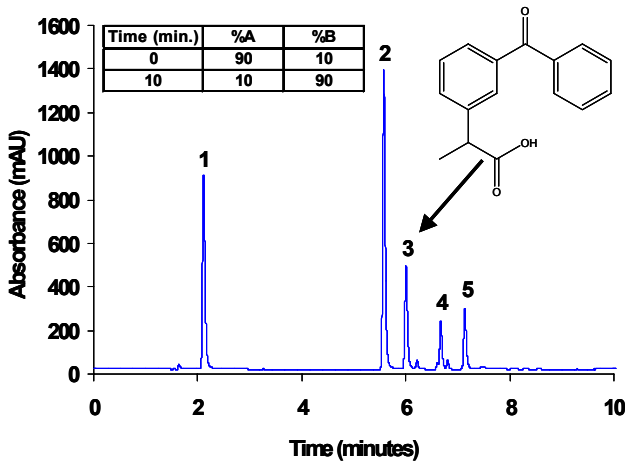

Figure 3. Separation of (1) Acetaminophen, (2) Naproxen, (3) Ketoprofen, (4) Fenoprofen, and (5) Indomethacin on ZirChrom®-EZ, 150 mm x 4.6 mm i.d. (P/N, EZ01-1546); Mobile Phase, A: 20 mM ammonium acetate, pH 5.0 B: acetonitrile; Temperature, 35 ºC with Metalox™ 200-C Column Heater; Flow rate, 1.0 ml/min.; Injection volume, 10 µl; Pressure drop, 168 bar; Detection by UV at 254 nm.

Enhanced Retention of Basic Drugs on ZirChrom®-EZ

Another feature of the ZirChrom®-EZ phase is its relatively higher retention for basic drugs in LC/MS compatible mobile phases compared to ZirChrom®-PBD. Figure 4 shows that under gradient elution with ammonium acetate buffer at pH 6.0, ZirChrom®-EZ gives significantly higher retention for the basic compounds metanephrine and normetanephrine than does ZirChrom®-PBD under the same conditions. Based on these results, isocratic conditions can be used for the separation of

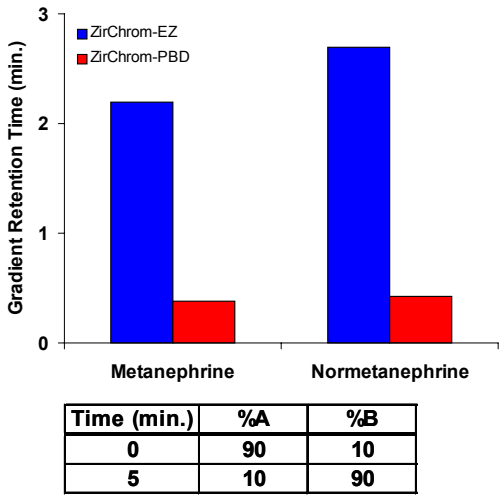

Figure 4. Gradient retention time of metanephrine and normetanephrine on ZirChrom®-EZ versus ZirChrom®-PBD, where A = 20mM ammonium acetate, pH 6.0, and B = acetonitrile.

metanephrine and normetanephrine on ZirChrom®-EZ as demonstrated in Figure 5, where excellent resolution is obtained in less than 5 minutes using only a 5 cm long column.

ZirChrom®-EZ Exhibits Unique Selectivity for Basic Pharmaceuticals

As a result of the mixed-mode ion-exchange and reversed-phase characteristics of ZirChrom®-EZ, the elution order of basic pharmaceuticals is often quite different compared to leading ODS phases. Figure 6 shows a plot of log k' for eleven common antihistamines and antidepressants on a leading ODS phase versus log k' for the same compounds on ZirChrom®-EZ. There is no apparent correlation of the retention for these compounds on the ODS phase with the retention on ZirChrom®-EZ. This different selectivity is particularly useful in method development for basic pharmaceuticals. When a pair of basic compounds cannot be separated using a traditional ODS phase, the chances of them separating on ZirChrom®-EZ are much better than on another ODS phase. As a way of demonstrating the utility of this different selectivity, we have investigated the traditionally difficult separation of morphine and codeine from their isomeric metabolites hydromorphone and hydrocodone. Figure 7 shows that ZirChrom®-EZ makes this separation easy, providing baseline separation of all four compounds in less than five minutes.

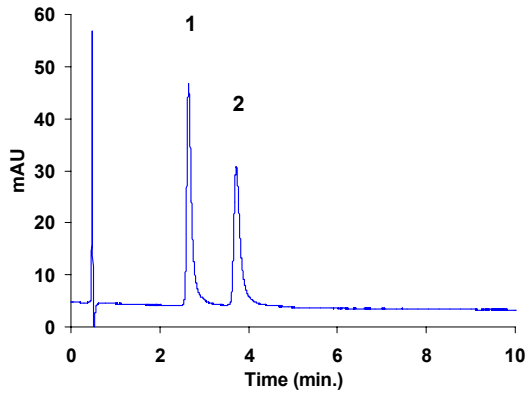

Figure 5. Isocratic separation of (1) metanephrine and (2) normetanephrine on ZirChrom®-EZ, 50 mm x 4.6 mm i.d. (P/N, EZ01-0546) in an LC/MS compatible mobile phase. **LC Conditions**: Mobile phase, 25/75 ACN/20mM ammonium acetate, pH 6.0; Flow rate, 1.20 ml/min.; Temperature, 35 ºC with Metalox™ 200C; Injection volume, 10 µl; Detection at 254 nm.

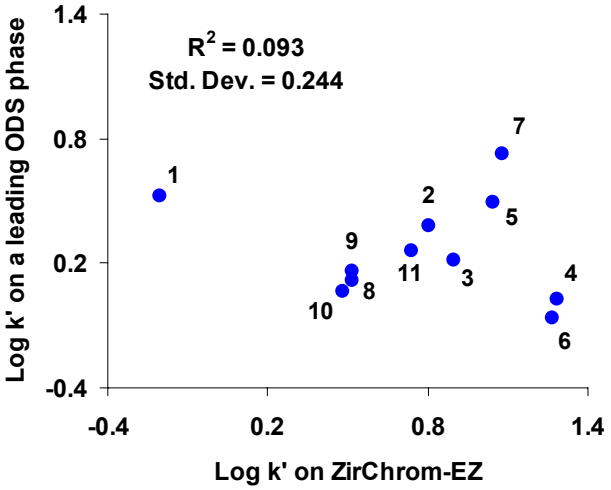

Figure 6. Plot of log k' for several antihistamine and antidepressant drugs on a leading ODS phase versus log k' for the same compounds on ZirChrom®-EZ. **LC Conditions**: Mobile phase, 72/28 MeOH/25mM Ammonium phosphate, pH 6.0; Flow rate, 1.0 ml/min.; Temperature, 35 ºC with Metalox™ 200C; Injection volume, 5 ml; Detection at 254 nm; Solutes: 1=hydroxyzine, 2=thiothixene, 3=doxepin, 4=nortryptyline, 5=amitryptyline, 6=desipramine, 7=perphenazine, 8=pyrilamine, 9=tripelennamine, 10=methapyrilamine, and 11=brompheniramine.

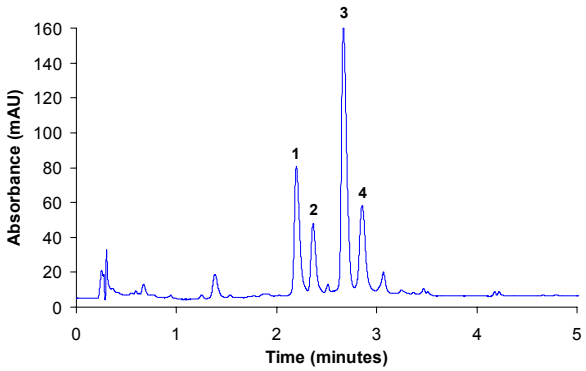

Figure 7. Separation of abused opioids and their metabolites on ZirChrom®-EZ, 50 mm x 4.6 mm i.d. (EZ01-0546). **LC Conditions**: Mobile phase, gradient elution from 10-90% B from 0-5 minutes, with A = 20mM ammonium acetate, pH 6.0, B = ACN; Flow rate, 2.00 ml/min.; Temperature, 35 ºC with Metalox™ 200C; Injection volume, 10 µl; Detection at 254 nm.; Solutes: 1=Morphine, 2=Hydromorphone, 3=Codeine, and 4=Hydrocodone.

Summary

Here we report the introduction of a first of its kind Lewis acid deactivated zirconia-based reversed-phase HPLC column, which is easier to use and less prone to problems due to solute interactions with the strong Lewis acid sites on the surface and which still has the inherent chemical stability advantage of zirconia. Most importantly this new column still maintains the very different chromatographic selectivity for basic pharmaceuticals that zirconia-based columns are known to have compared to traditional bonded silica phases. This new column compliments the four reversed phase columns that ZirChrom currently sells, which fill the need for chemically different and thermally stable HPLC supports.

Visit www.zirchrom.com for more application notes using ultra-stable, high efficiency ZirChrom® columns.

References

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