Analytical Advantages of Highly Stable Stationary Phases for Reversed-Phase Liquid Chromatography

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ABSTRACT

Over the past five years, many of the top manufacturers of columns for high performance

liquid chromatography (HPLC) have focused on improving stationary phase stability and

reproducibility. Improved column stability--both chemical and thermal--offers new

advantages, such as decreased analysis time, and new methods of selectivity optimization.

Stabler HPLC packing materials have been achieved through advances in silane

chemistry; however, the greatest improvements in stability have resulted from the use of

alternative non-silica supports such as synthetic organic polymers, alumina, and zirconia.

In this study, the authors use a wide variety of test solutes to compare the efficiency,

selectivity, and hydrophobic retention mechanisms of five commercially available

reportedly "stable" HPLC columns based on silica (ODS silica), alumina (PBD coated

alumina), zirconia (Zirchrom-PBD and Zirchrom-CARB, Zirchrom Separations), and

polystyrene cross linked with divinylbenzene (PS-DVB) as the support. Here we define

"stable" as having enhanced stability from a "conventional" ODS silica column that has a

working pH range generally from pH 2-8. The polybutadiene (PBD)-coated zirconia

column proved to be the most efficient of the five, whereas the polymeric phase was

markedly less efficient than the other four. When compared to a conventional silane-

modified silica column, the PBD-coated zirconia, PBD-coated alumina, and polymer

columns were similar with respect to reversed-phase chemical selectivity, whereas the

carbon-clad zirconia column exhibited the greatest differences in selectivity for the

primarily non-electrolyte molecular probes tested. Thus, while all four of the non-silica

columns are stabler than silica, the Zirchrom-CARB column should be used to obtain the

largest changes in chromatographic selectivity when a separation cannot be achieved on a more "traditional" reversed-phase silica column. All but the polymer phase were found to have similar hydrophobicities as assessed through the free energy of retention of a methylene group. Finally, a principal component analysis of the retention data for 21 test analytes shows that compared to data obtained for a group of silane-bonded silica phases, the five phases under study differ markedly in their mode of retention.

INTRODUCTION

Reversed-phase (RP) continues to be the dominant mode of high performance liquid

chromatography (HPLC) due to the wide range of chemically diverse compounds that can

be analyzed by this technique and the large body of literature on RPLC which greatly

facilitates the development of new analyses. Historically, silica gel has been the support

material of choice for producing RP stationary phases. Silica's flexible chemical surface

allows for its ready modification through a diverse range of silane chemistry. In addition,

its wide range of accessible particle and pore sizes, monodispersity, mechanical stability,

and good mass transfer properties have promoted silica's dominant position in liquid

chromatography.

Analytical advantages of pH and thermal stability: Recent research on silica-based

stationary phases has largely focused on exploiting the bonding chemistry and improving

the chemical and thermal stability of the base silica (1-2). This work is rooted in

practicality: enhanced pH and thermal stability of RPLC packing materials allows for the

widest possible range of chromatographic conditions to be exploited by the

chromatographer (see Figure 1). For example, high pH can be used to suppress amine

protonation, while low pH can be used to suppress the ionization of acidic solutes.

Extreme chemical stability also permits the cleaning of a "fouled" column under very

acidic conditions or the sterilization (depyrogenation) of a column with alcoholic basic

solutions. The retention of anions by protonating them can be increased by the use of low

pH, thereby avoiding the need for quaternary amine ion pairing agents. Similarly, the

retention of positively charged amines can, in principle, be increased by raising the pH

4

above the pKa of the protonated Brönsted acid. Unfortunately, the pH required to fully protonate many carboxylic acids and completely deprotonate aliphatic amines are outside the range of accessible pH's (pH 2-8) of silica-based phases (3).

Thermal column stability has its own distinct inherent advantages (see Figure 1). It can allow for analysis at higher column temperatures which lowers mobile phase viscosity and lessens the mechanical wear and tear on the LC pumping system. Higher run temperatures also lower the retention of solutes in RP mode, which can often result in a faster analysis or the ability to lower the amount of organic modifier needed to elute the solutes. In their seminal paper, Antia and Horvath (4) carried out a series of detailed analytical calculations regarding the potential benefits of the use of high temperature to bring about significant, even tenfold reductions in analysis time. Their work predicts a very considerable decrease in time of analysis with an increase in column temperature, while maintaining a constant pressure drop by increasing flow rate or decreasing the column length. Of course, analysis speed is also limited by the decrease in plate count at higher linear velocities. Here again, the thermal stability of a column is beneficial: an increase in column temperature actually helps to improve the column efficiency at high velocity by increasing the rate of solute diffusion into and out of the stationary phase particle, thereby decreasing the peak width (5-6).

With thermal stability comes the freedom to use column temperature to optimize the separation; this can lead to a more robust separation or to beneficial selectivity changes. For example, recently Snyder and coworkers (7-8) showed that temperature, when used in conjunction with adjustments in mobile phase composition, can be a very powerful aid in optimizing separations even with stationary phases whose upper

temperature limits are only 80 - 90°C. Thermal stability can also make method

development easier since temperature is easily changed, whereas mobile phase changes

may require long equilibration periods upon switching between drastically different

organic modifiers.

Another driving force for the recent interest in high temperature HPLC is the

concern for doing "green chemistry" and the minimization or elimination of toxic waste

generation. Building on Hawthorne, Yang, and Miller's (9) work using supercritical

water, Smith and Burgess (10, 11) used sub-critical (up to 210°C) water with no organic

modifier to separate both polar and hydrophobic species including priority phenols and

drugs on polymer reversed-phase media. They showed that UV detection could be done

even at very short wavelength (190nm), and thus many species which do not absorb at

longer wavelength can be easily detected. Similarly, Miller and Hawthorne (12) showed

that an FID detector can be used with pure water as the eluent. The boiling point of water

is about 200°C at 20 bar, so only a small back pressure is needed to prevent boiling. An

ODS column, however, proved to be too unstable even in pure water at temperatures of

only 120°C to be used on a practical time scale. Indeed, the typical commercial alkyl

silane bonded silica phase is seldom used at more than 20 - 30° C above room

temperature due to its instability at higher temperatures (13-22), especially in phosphate

buffers.

The advent of stabler phases for RPLC: Recent advances in silane chemistry

have led to the development of stabler silica-based phases (1-2); however, even greater

strides in stability--both thermal and chemical--have been achieved through the use of

alternatives to silica gel such as synthetic organic polymers (15-18), alumina (13,14, 23-

24), and zirconia (25-40). Zirconia-based columns have received a great deal of attention recently due to their extraordinary stability under extreme thermal and chemical conditions (41-43). Zirconia's enhanced chemical stability compared to other metal oxides is illustrated in Figure 2 (44) which shows no detectable dissolution of zirconia over the entire pH range over 15 days of exposure, whereas a significant amount of alumina is dissolved under the same conditions. The chemical stability contrast between zirconia and silica would be even greater, as silica is more soluble than alumina above neutral pHs.

When coated with a thin layer of polybutadiene (PBD), zirconia becomes a reversed-phase (ZirChrom-PBD) able to withstand extended exposure to mobile phases at pH 14 under flow conditions (Figure 3A), as well as column temperatures as high as 200°C (Figure 3B). The extraordinary thermal stability of this column allows for the very rapid analysis of a series of chlorophenols at 200°C in a purely aqueous mobile phase (Figure 4). At low column temperature (30°C), an organic modifier is required to elute the chlorophenols from the column (Figure 4A). As the column temperature is increased to 80°C, the retention time of the last eluting solute is more than halved at the same flow rate (Figure 4B), and at 200°C the separation is achieved both quickly and without any organic modifier (Figure 4C).

As a final illustration, Figure 5 shows the separation of a series of tricyclic antidepressants on a zirconia-PBD phase compared to that on a conventional silica-ODS phase. The chromatogram acquired on the zirconia column shows much better peak symmetry and efficiency. Moreover, the separation on the Zirchrom-PBD column was

achieved in less than half the time as on the silica column, and at a pH of 12 which is inaccessible on conventional silica-ODS phases.

With the continuing drive by HPLC column manufacturers to develop stabler and more reproducible stationary phases, a comparison of performance properties will benefit the chromatographer faced with choosing from among this expanding array of robust columns. In this work, we examined the RP selectivity and efficiency of five of the most stable types of currently available RP stationary phases: a prototypical silica-based phase (ODS silica), a purely polymeric phase, two polymer coated metal oxide phases—one based on alumina and one on zirconia (ZirChrom-PBD, ZirChrom Separations)—and lastly a graphitized carbon clad zirconia phase (ZirChrom-CARB, ZirChrom Separations) that has very similar chromatographic selectivity to Hypercarb (45). We also looked more closely at the retention mechanisms of these five phases by examining the hydrophobic interactions of each with a homolog series of alkylbenzenes and by performing a principal component analysis of retention data for 21 test analytes.

EXPERIMENTAL

The general characteristics of the five columns under study, including particle and pore size, column dimensions, low and high pH limits, temperature limits, manufacturer, and cost (at the time the study was done) are given in Table I. To assess their RP selectivity and efficiency, twenty-seven probe analytes were chosen to cover as wide a range of solute-stationary phase interactions as possible: 1=uracil, 2=benzyl formamide, 3=benzyl alcohol, 4=phenol, 5=benzoic acid, 6=3-phenyl propanol, 7=acetophenone, 8=benzonitrile, 9=p-chlorophenol, 10=methyl benzoate, 11=nitrobenzne, 12=anisole,

8

13=benzene, 14=pyridine, 15=p-nitrotoluene, 16=N, N-dimethylaniline, 17=p-nitrobenzyl

chloride, 18=toluene, 19=4-butylbenzoic acid, 20=benzophenone, 21=bromobenzene,

22=ethylbenzene, 23=p-xylene, 24=naphthalene, 25=p-dichlorobenzene,

26=propylbenzene, 27=butylbenzene.

Mobile phase conditions for the efficiency and selectivity experiments were

chosen to be similar to those employed in a previous study (46) which tested 86 different

silica-based RP columns with 6 probe analytes. The mobile phase was 40/60 ACN/buffer

(50 mM phosphate buffer at pH 3.2) with a flow rate of 1.0 mL/min, a column

temperature of 21°C, and UV detection at 254 nm. All chemicals were reagent grade or

better. All chromatograms were collected on a Hewlett-Packard 1100 chromatograph

(Palo Alto, CA, USA) with a variable wavelength UV detector and a Hewlett-Packard

ChemStation for data collection. Uracil served as the dead volume marker for all

chromatographic investigations.

Principal component analysis was performed using the singular value

decomposition function from Matlab 5.2. To remove differences in log k' values caused

by the unique phase ratio of each column, the log k' values from each column were mean-

centered before the analysis. The number of principal components was determined by

comparing the residual standard deviation to the estimated experimental error in the log k'

values (~0.02 unit in log k'). For details concerning principal component analysis, the

reader is referred to the literature (47).

RESULTS AND DISCUSSION

Column Efficiency: The number of theoretical plates for all twenty-seven test analytes on each of the five columns is shown in Table II. Each column type is characterized by two global figures of merit: the average plate count over all analytes and the median plate count over all analytes. The ZirChrom-PBD and silica based columns proved to be equally efficient overall for these 27 probe molecules, with both having a mean reduced plate height of 5.5. The alumina-based phase, ZirChrom-CARB, and the polymeric phase had mean reduced plate heights of 6.8, 19 and 26, respectively.

Each column exhibited a fairly wide range of plate counts for the different solutes, but the polymer phase gave consistently and considerably lower plate counts that either the silica-, alumnia-, or zirconia-based phases. Also note that the PBD-coated zirconia phase showed both the best reduced plate height for the maximum and minimum plate count compared to all other phases. This very high efficiency results in part from the small (3μ), highly monodisperse size of these particles, as well as the fast solute mass transfer that can occur within the thin layer of cross-linked PBD as opposed to a purely polymeric phase. In Figure 6, the chromatographic efficiency of the Zirchrom-PBD column is compared to that of two purely polymeric phases for the separation of three alkylparabens. While in general polymeric columns share the pH stability of zirconia-based columns, they have poor column efficiency even for small molecules such as these.

Chromatographic Selectivity: Next, we investigated differences in retention selectivity for the 27 probe analytes on the five test columns. Specifically, we compared their k′ values on the silica-based column to their selectivity on the other four. Although it is true that end-capping, carbon load, and purity of silica do have an effect on retention and band spacing for different bonded-phase ODS silica columns, it rarely leads to major changes

in band spacing of nonelectrolytes as long as all other chromatographic conditions are the same. Thus, we believe our comparison of selectivity differences between these five stable columns will be indicative of global differences between the different substrates in relation to most silica-based C18 RP materials.

Based on the manufacturers' data, we anticipated a fairly wide range in the amount of stationary phase, and thus phase ratios, among these different materials, which would be reflected in a broad range of k′ values. To normalize the phase ratios, the selectivity data in Figure 7 are presented as the ratio of k′ for any given solute to that of benzene. The ZirChrom-PBD and the silica-based column show very similar chemical selectivity for this chemically diverse range of probe solutes (Figure 7A). The main differences in selectivity are found for analytes 14 (pyridine) and 16 (N,N-dimethylaniline). Both of these compounds are basic and positively charged under the experimental mobile phase conditions, and it is well known that the adsorption of phosphate from the eluent imparts a negative charge to the surface of zirconia-based stationary phases. This results in greater retention of positively charged species on the ZirChrom-PBD column due to mixed-mode RP and cation-exchange retention processes (34-35). Recent work at ZirChrom Separations show that the loading capacity of ZirChrom-PBD for positively charged drugs is three to four-fold greater than many conventional columns.

Similarly, the alumina-based phase shows very similar selectivity to the silica-based phase under these conditions (Figure 7B); however, the overall retention on the PBD coated alumina column is very low, which is likely due to a much lower stationary phase loading. As for the ZirChrom-PBD and PBD coated alumina, adsorption of

11

phosphate onto the surface through a Lewis acid-base reaction induces a net negative

charge on the phase (35), which leads to the high retention of cationic pyridine and N,N-

dimethylaniline. Figure 7C illustrates the more varied retention properties of the PLRP-S

polymer phase compared to those of the silica phase. While the general trend of retention

is similar on both columns, the elution order is often different. Finally, Figure 7D

compares the selectivity of the test solutes on the ZirChrom-CARB column versus the

silica-based column. Of the four columns tested, this graphitized carbon clad zirconia

phase has the most markedly different selectivity from that of the silica phase. This

result is as expected, in that the column's graphite-like layer stationary phase is rigid and

cannot accommodate partitioning into a bonded phase or polymeric stationary phase

matrix (45). One practical outcome of this is illustrated in Figure 8, which shows the

successful separation of two aromatic diastereomers on the Zirchrom-CARB column.

This separation cannot be achieved on a conventional silica-ODS column, despite its

higher efficiency. The inability of solutes to partition into the rigid carbon phase gives

rise to its chemical selectivity for different geometric isomers (42).

The selectivity differences between the various phases and their global similarity

to the ODS-type phase are shown from a different perspective in the log k'-log k' plots in

Figure 9. Such plots were developed by Horvath (46) to study similarities in retention

energetics on different types of reversed phases. High correlation coefficients and slopes

close to unity indicate near identity of retention thermodynamics for the two phases. The

r^2 values of 0.980 and 0.978 for the Zirchrom-PBD and PBD-coated alumina,

respectively, show that they are most similar to the C18 silica phase with respect to

retention processes. The very weak correlation ($r^2 = 0.529$) between the Zirchrom-CARB

and silica column show that they are most dissimilar, with polymer phase occupying a position intermediate between the polymer-coated metal oxides and the carbon-coated zirconia. Note that the amine solutes were not considered in the analysis.

Hydrophobic Interactions: One of the key characteristics that classifies a particular material as a reversed-phase and which can be used to distinguish between different reversed-phase materials is the existence of a linear relationship between log k' and the number of methylene units in a homolog series. Such data are presented in Figure 10 for a series of n-alkylbenzene homologs which serve as prototypical hydrophobic (non-polar) analytes. The plots indicate that absolute retention of these non-polar solutes differs greatly among the five phases. Clearly, retention is greatest on the polymeric phase followed by the C18 silica phase, the Zirchrom-CARB, and the Zirchrom-PBD phases. Retention on the polymeric PBD coated alumina phase is very low, supporting our statement above that there is only a small amount of polymer present on the material.

However, absolute retention as represented by the vertical position in Figure 10 and the magnitude of k' is not necessarily the best index of phase hydrophobicity. Since the free energy of retention can be related to k' as:

$$\Delta G^o = -RT * \ln (k'/\phi) \tag{1}$$

(where R is the gas constant and T is the temperature in Kelvin), the slopes of the linear regression lines in Figure 10 are proportional to the free energy of transfer of a methylene group from the mobile phase to the stationary phase. This is shown directly in equation 2:

$$\Delta G^o_{\text{retn, } CH_2} = -RT * \ln (k'_{n+1}/k'_n) \tag{2}$$

where k'_{n+1} and k'_n denote the capacity factors of the nth and $n^{th} + 1$ homologs. These slopes for each of the five "stable" stationary phases are as follows: -0.583 (polymeric), -0.880 (C18 silica), –0.887 (Zirchrom-CARB), –0.826 (Zirchrom-PBD), and -0.906 (PBD coated alumina) kJ/mole. Thus, all but the polymer phase have similar hydrophobicities as assessed through the free energy of retention of a methylene group.

Principal Component Analysis of Retention Factor Data: Lastly, we conducted a principal component analysis (PCA) of the retention data to fully compare retention on these five stable phases--both among themselves and as compared to five conventional silica-based bonded phases (e.g., C18 and C8) for which a PCA has been previous reported (48). The PCA analysis does not impose any preconceived model of retention. Analytes 1, 2, 5, 14, 16 and 19 were omitted to allow a direct comparison with the results from the previous silica study. The results of the analysis show that four principal components are required to describe 99.96% of the variability in the retention data set (see Table III). In contrast, results from the study of five silica-based RP columns show that only one principal component is required to describe 99.92% of the total variance in the retention factor data (48). Moreover, in this previous study all of the correlation coefficients for the log k' versus log k' plots were 0.997 or better, indicating very similar retention behavior for the same 21 probe molecules on these silica columns. The differences among the five "stable" columns in the present study are even more apparent when the columns are plotted using the scores of the first two principal components, with the ZirChrom-CARB and PBD coated alumina columns emerging as the most distinct

(see Figure 11). Taken together, the PCA results suggest that intermolecular interactions

for the analytes on the five "stable" chemically distinct columns in this study differ from

one another in a more complex way than they do on the conventional RP silica phases.

CONCLUSIONS

Advances in the chemical and thermal stability of HPLC column packing materials allow

for the use of mobile phase conditions and column temperatures that have hither to been

unreachable with silica-based stationary phases. The practical benefits of these more

stable chromatographic materials range from the ability to use harsh cleaning conditions

for column regeneration, to increased analysis speeds up of to an order of magnitude or

better. Moreover, the amount of organic waste generated can be greatly reduced or even

eliminated at elevated column temperatures.

Our comparison of five distinct "stable" columns exhibiting varying degrees of

chemical and thermal stability showed that the ZirChrom-PBD column is the most

efficient, and the polymeric the least efficient. In terms of chromatographic selectivity for

nonelectrolytes, the ZirChrom-PBD and PBD coated alumina columns were very similar.

In contrast, the ZirChrom-CARB column showed the greatest difference in RP chemical

selectivity of any of the columns tested compared to the silica ODS column. Practically

speaking, the ZirChrom-CARB column should be used to get the largest change in

reversed-phase chemical selectivity when a separation does not work on a more

"traditional" reversed-phase material. The PCA study revealed that four principal

components are necessary to describe retention on the five "stable" columns, compared to

one principal component for five analogous reversed-phase silica columns. This suggests

a more complicated mode of retention for this next generation of stable HPLC columns.

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FIGURE CAPTIONS

Figure 1: Benefits of extraordinary chemical (pH) and thermal stationary phase stability for reversed-phase HPLC analyses.

Figure 2: Comparison of pH stability of zirconia stationary phase and alumina phase as analyzed by ICP (44).

Figure 3: Chemical (A) and thermal (B) stability of 150 x 4.6 mm i.d. ZirChrom-PBD column, flow rate, 1.0 ml/min. (A): wash fluid = 90/10 1M NaOH in water/MeOH (pH 14), mobile phase = 50/50 ACN/water, temperature = 35°C. (B): mobile phase = 15/85 ACN/water, temperature = 195°C.

Figure 4: Separation of phenol and four of its chloroderivatives on a 150 x 4.6 mm i.d ZirChrom-PBD column, flow rate = 3.0 ml/min, 254 nm detection; 1=phenol, 2=4-chlorophenol, 3=4-chloro-3-methyl phenol, 4=2,4,6-trimethylphenol, and 5=2,4,6-trichlorophenol. (A): mobile phase = 65/35 water/ACN, temperature = 30°C. (B): same mobile phase as (A), but temperature = 80°C. (C): mobile phase = 100% water, temperature = 200°C.

Figure 5: Separation of five tricyclic antidepressants on (A) ZirChrom-PBD (mobile phase = 45/55 ACN/20 mM potassium phosphate, pH=12.0) and (B) conventional silica-ODS phase (mobile phase = 50/50 ACN/20 mM potassium phosphate, pH=7). Flow rate = 1.0 mL/min, temperature = 30°C, detection at 254 nm, 1=Nordoxepin, 2=Protriptyline, 3=Nortriptyline, 4=Imipramine, 5=Amitriptyline.

Figure 6: Separation of three alkylparabens on (A) ZirChrom-PBD column (mobile phase = 10/10/20/60 MeOH/ACN/THF/water); (B) Polymeric column (mobile phase = 10/20/30/40 MeOH/ACN/THF/water; and (C) A different polymeric column than in B (same mobile phase as (B)). 150 x 4.6 mm i.d., flow rate = 1.0 ml/min, temperature = 50°C, injection volume = 2.0 μl, UV detection at 254 nm, 1=uracil (deadmarker), 2=ethylparaben, 3=propylparaben, and 4=butylparaben.

Figure 7: Selectivity comparison of normalized phase ratios (k' solute / k' benzene) between the C18 silica column (■) and each of the four non-silica reversed-phase columns: (A) ZirChrom-PBD (□), (B) PBD coated alumina (x), (C) Polymeric (◊), and (D) ZirChrom-CARB (○). Mobile phase = 40/60 ACN/50mM phosphate, pH 3.2, flow rate = 1.0 ml/min, temperature = 21°C, UV detection at 254 nm. Analytes 1-27 are identified in the Experimental section.

Figure 8: Separation of two aromatic diastereomers, (R)-Mosher-($\pm$)-Warfarin, on (A) 50 x 4.6 mm i.d. ZirChrom-CARB. Mobile phase = 45/55 THF/water, flow rate = 1.0 mL/min, ambient temperature, UV detection at 254 nm. The separation is unsuccessful on a 50 x 4.6 mm i.d. ODS column (B). Same experimental conditions as (A), except mobile phase = 40/60 THF/water.

Figure 9: k'- k' plots and associated regression statistics for comparison of retention thermodynamics between the ODS silica phase and the four non-silica phases. Mobile Phase = 40/60 ACN/50mM phosphate, pH 3.2, flow rate = 1.0 ml/min, temperature = 21°C, UV detection at 254 nm.

Figure 10: Plot of ln k' versus number of methylene units for a series of n-alkylbenzene homologs. □ = Polymeric column, ■ = ODS-silica, ○ = Zirchrom-CARB, ● =Zirchrom-PBD, and ▲ = PBD coated alumina. Mobile phase = 40/60 ACN/50mM phosphate, pH 3.2, flow rate = 1.0 ml/min, temperature = 21°C, UV detection at 254 nm.

Figure 11: Principal components analysis scores plot for the five test columns.
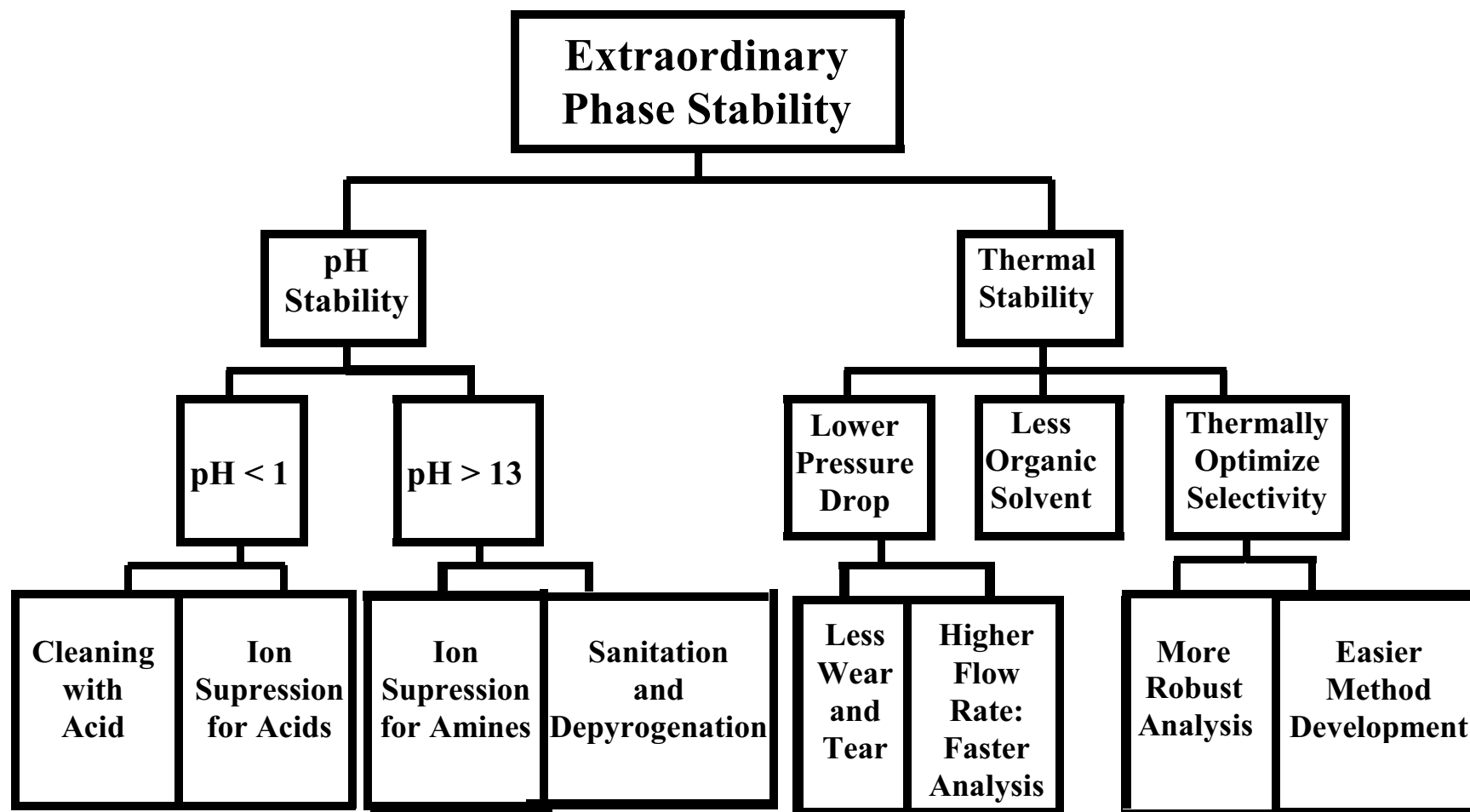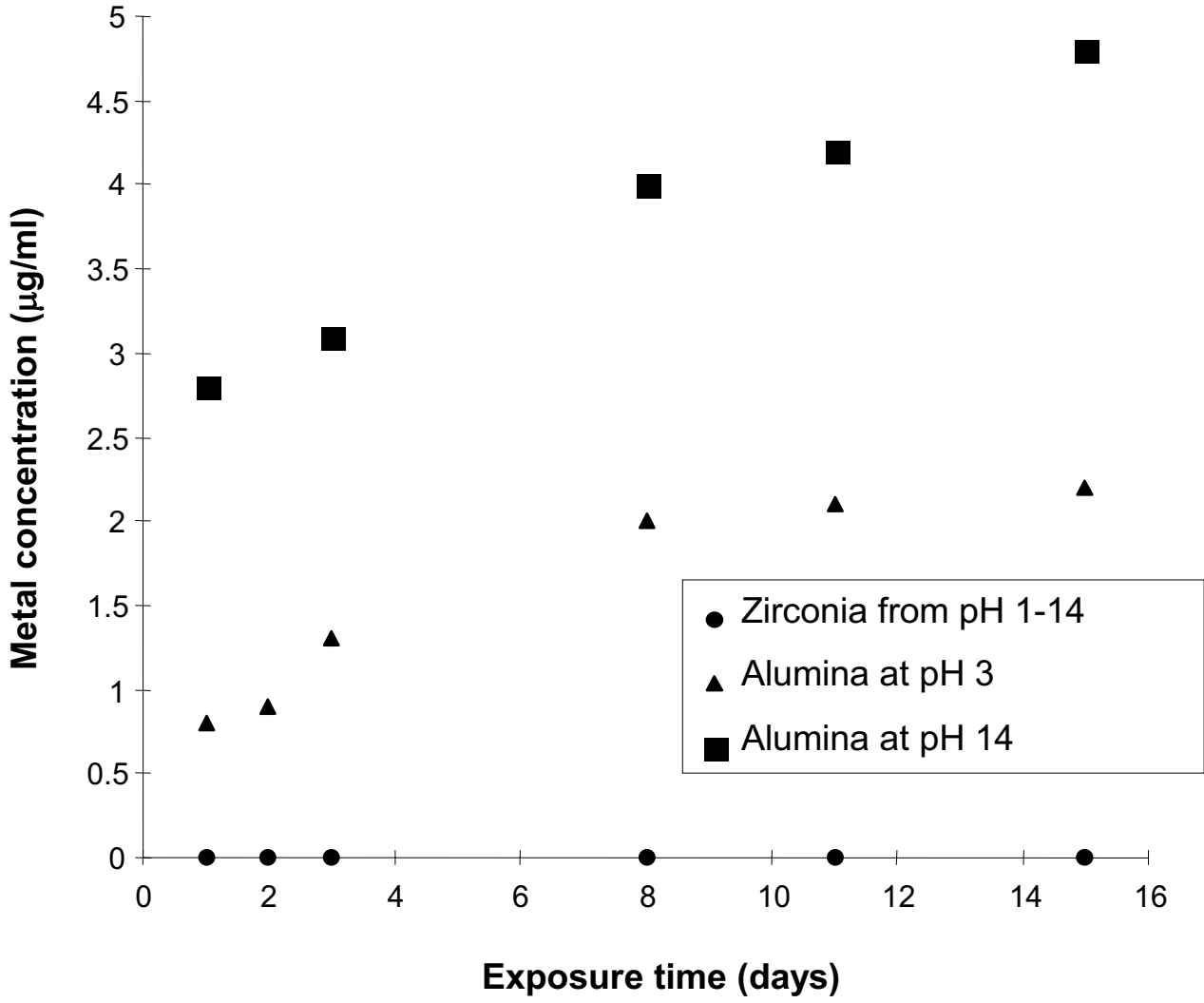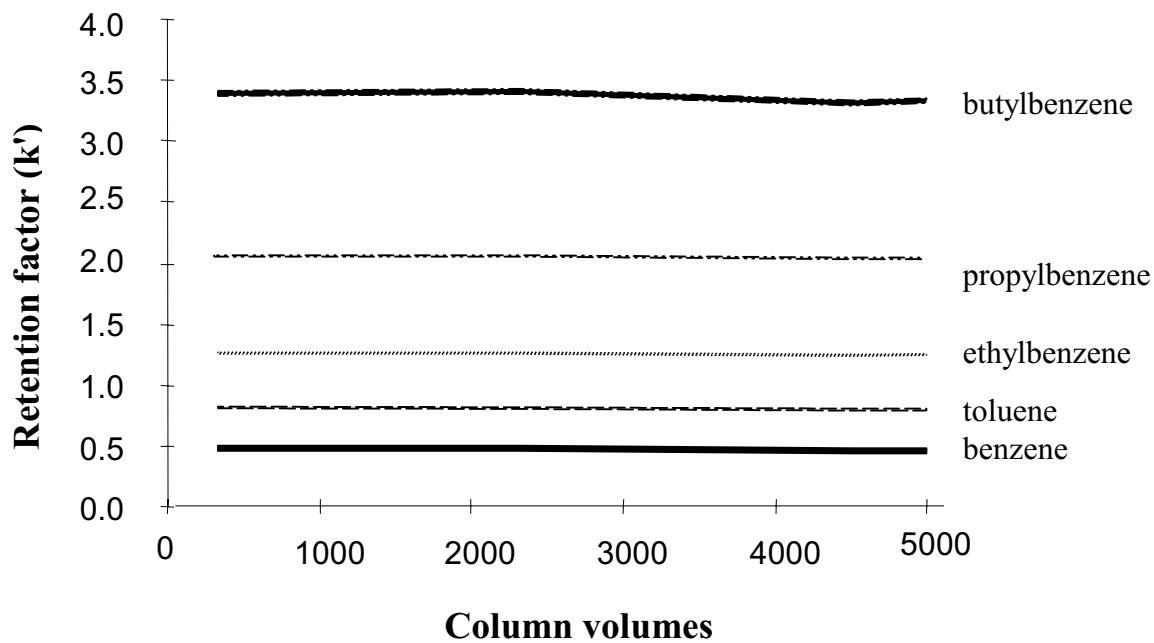
Figure 1

Extraordinary Phase Stability
- pH Stability
  - pH < 1
    - Cleaning with Acid
    - Ion Supression for Acids
  - pH > 13
    - Ion Supression for Amines
    - Sanitation and Depyrogenation
- Thermal Stability
  - Lower Pressure Drop
    - Less Wear and Tear
    - Higher Flow Rate: Faster Analysis
  - Less Organic Solvent
  - Thermally Optimize Selectivity
    - More Robust Analysis
    - Easier Method Development

Figure 2

Figure 3

(A) Chemical stability of Zirchrom-PBD column (pH = 14)

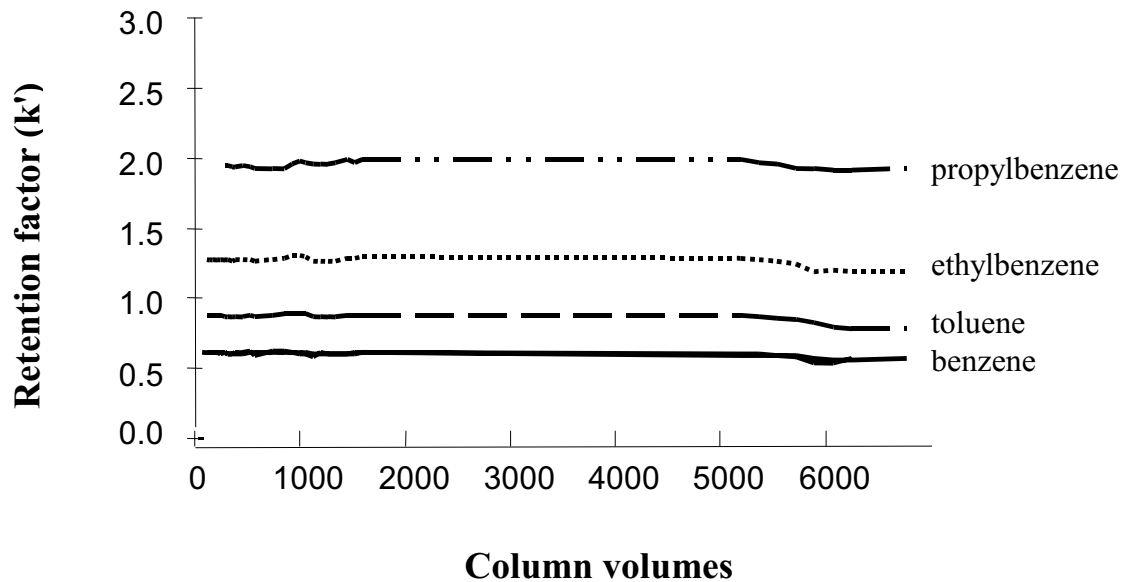## (B) Thermal stability of Zirchrom-PBD column (column temp = 195 °C)
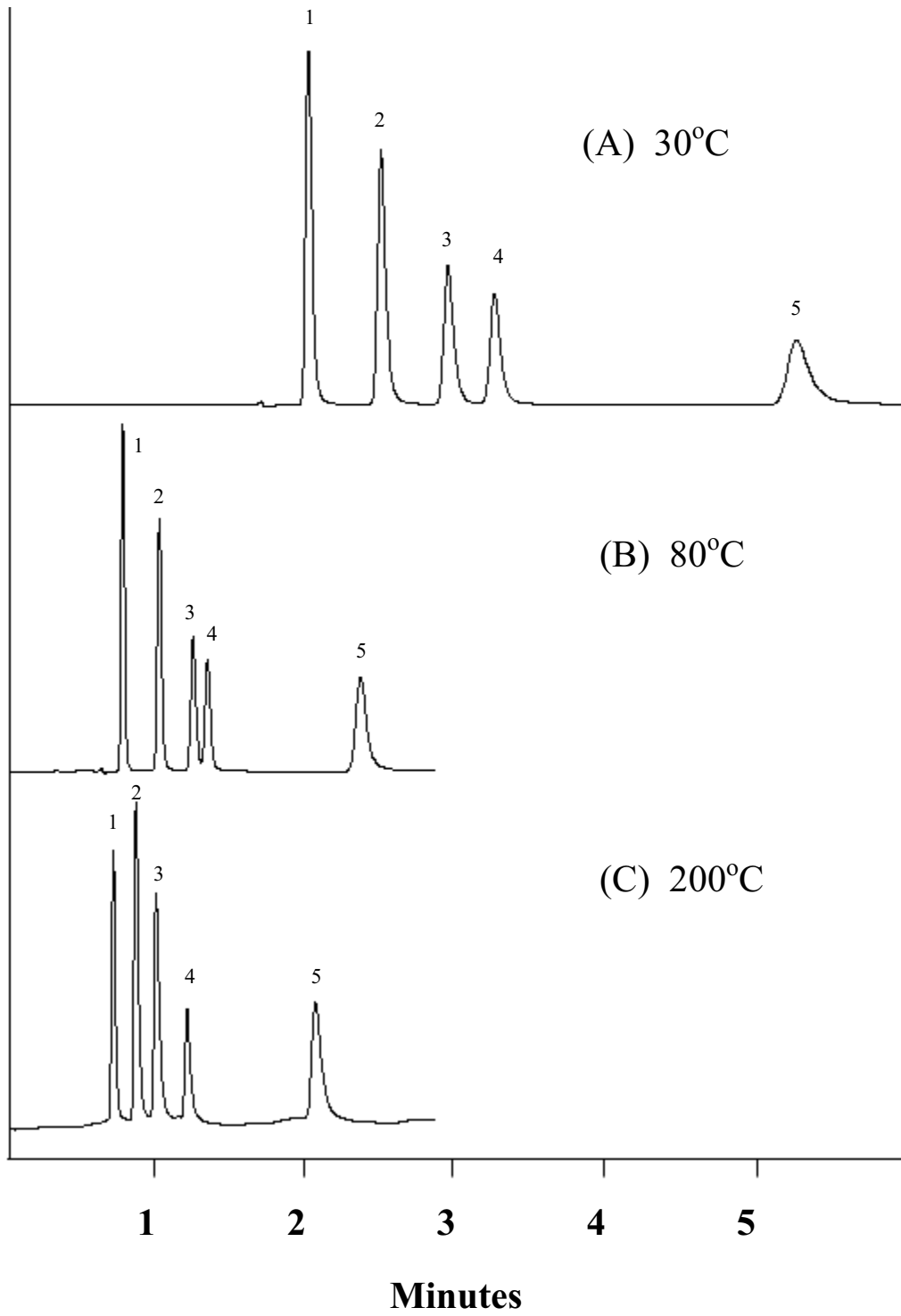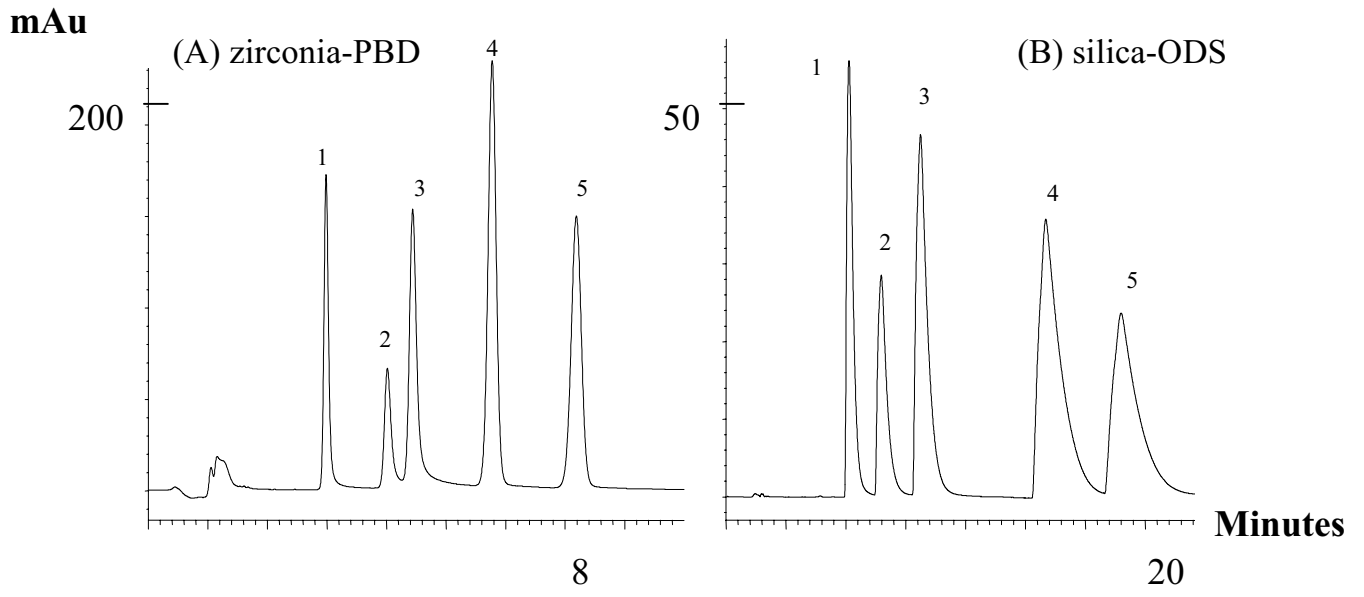
Figure 4

mAu

(A) 30°C

(B) 80°C

(C) 200°C

Minutes

24

Figure 5

mAu

200

(A) zirconia-PBD

1

2

3

4

5

50

(B) silica-ODS

1

2

3

4

5

8

20

Minutes

25

Figure 6.

(A) Zirchrom-PBD
1
2
3
4
N = 12,000

(B) Polymeric phase
1
2
3
4
N = 7,000

(C) Another polymeric phase
1
2
3
4
N = 1,000

Figure 7

(A) Zirchrom-PBD

(B) PBD-coated alumina

(C) Polymeric phase

(D) Zirchrom-CARB

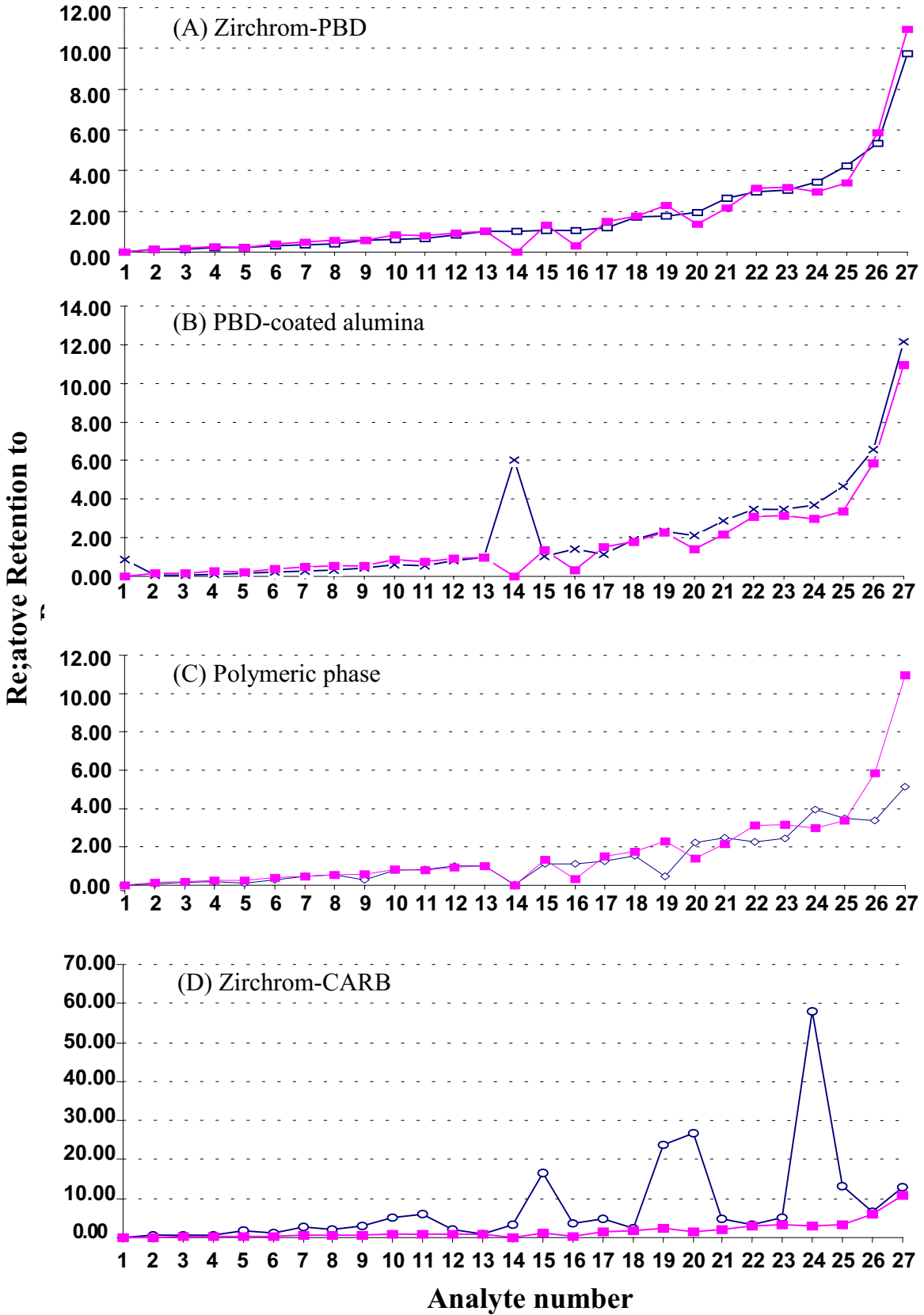Re;atove Retention to
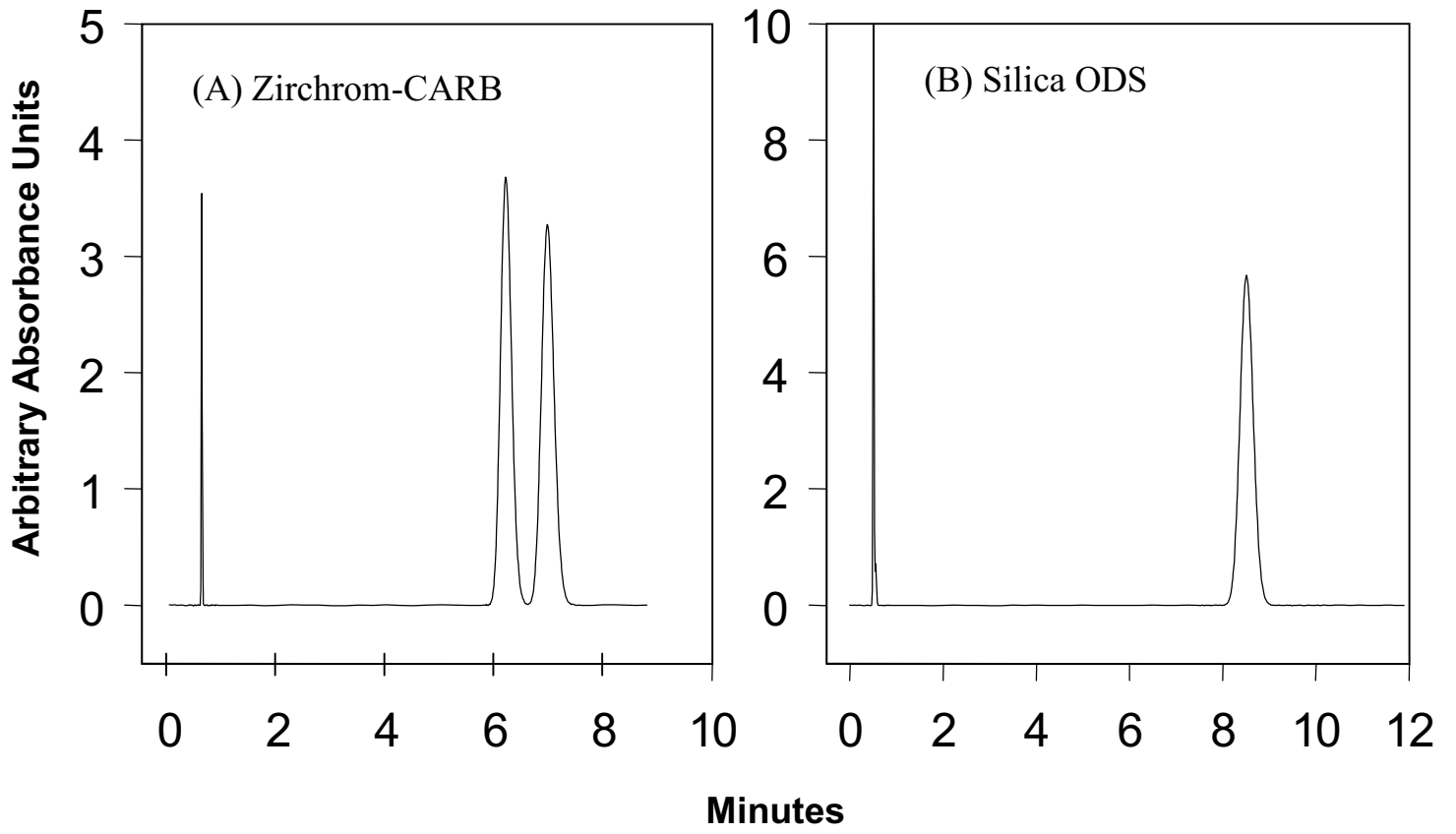
Analyte number
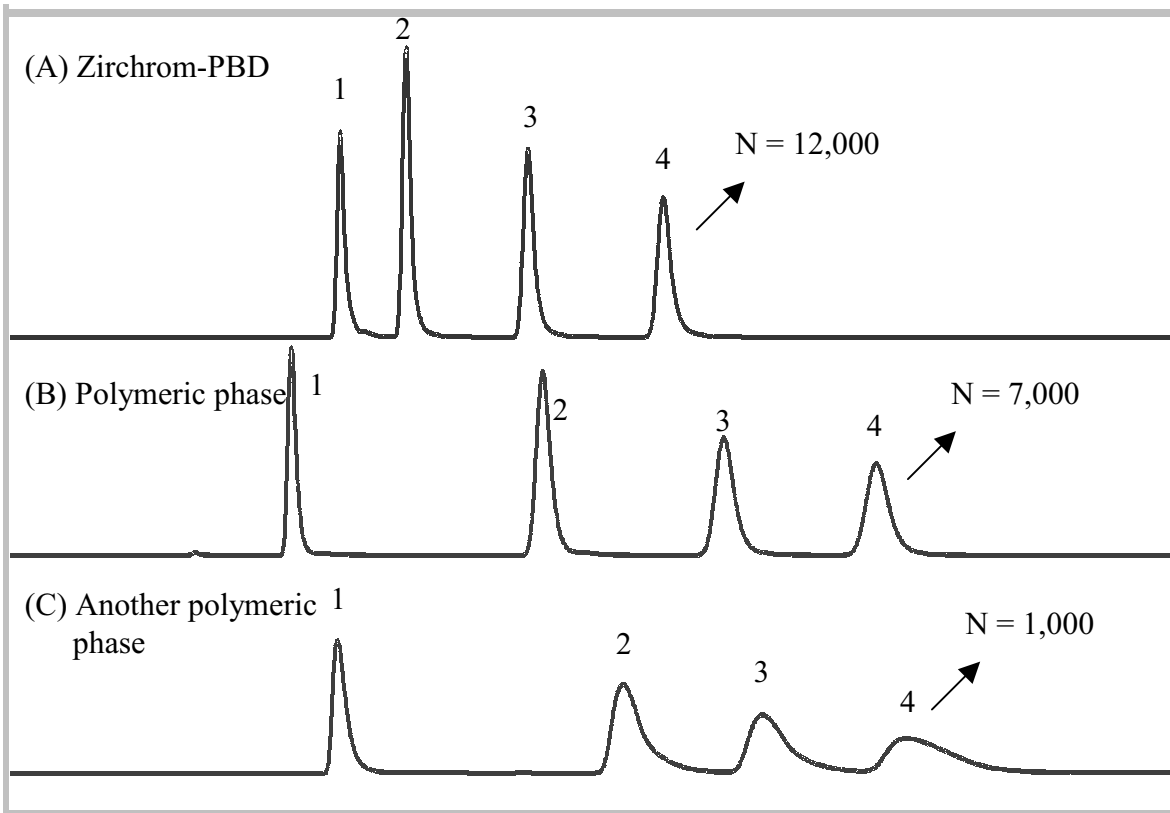
27

Figure 8

Figure 9

[**Note to Editor:** Figure 9 includes panels A-D and a small set of regression statistics. We have put each panel on a separate page to allow for the use of larger font sizes and thus promote better readability when these are reduced for publication. Please incorporate all four plots into a single figure when the manuscript is laid out. Alternatively, we can revise this figure to meet your requirements.]
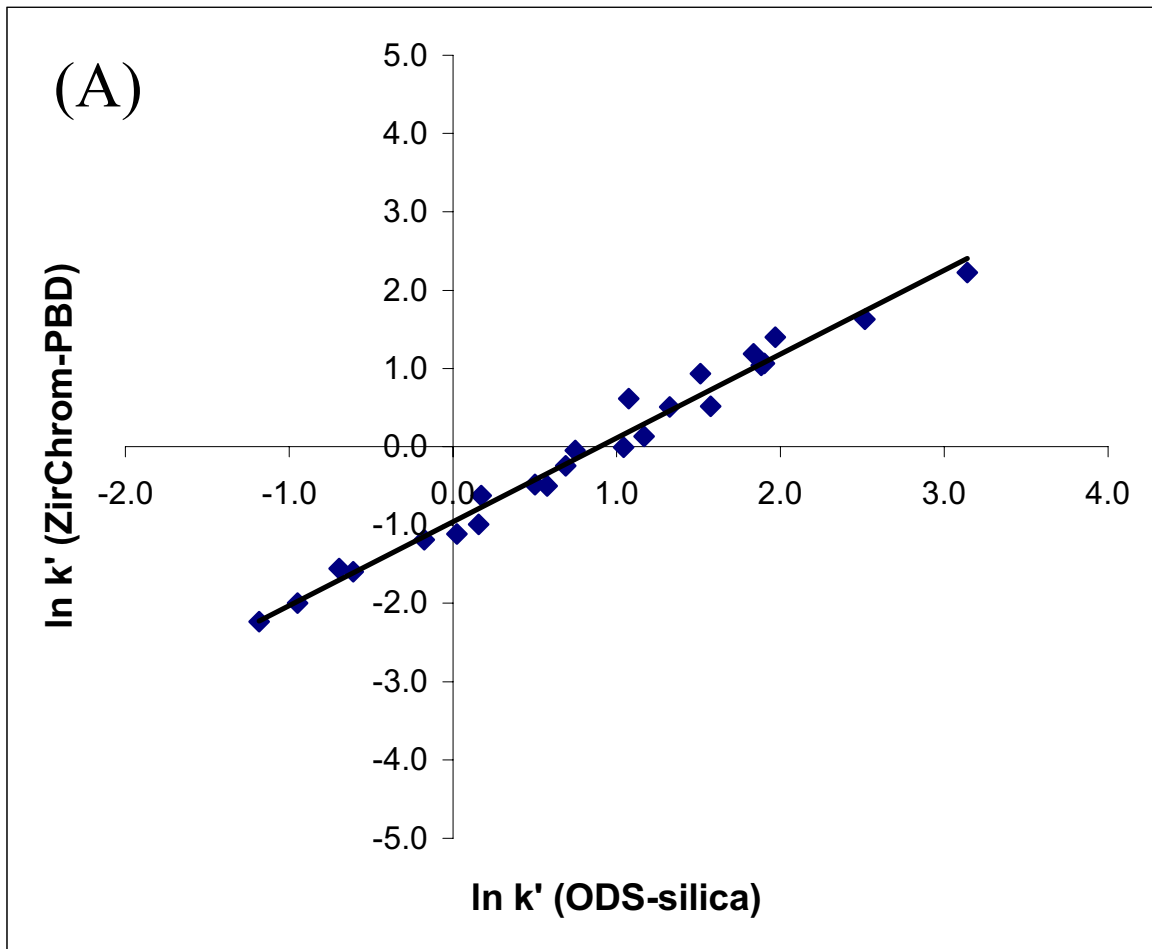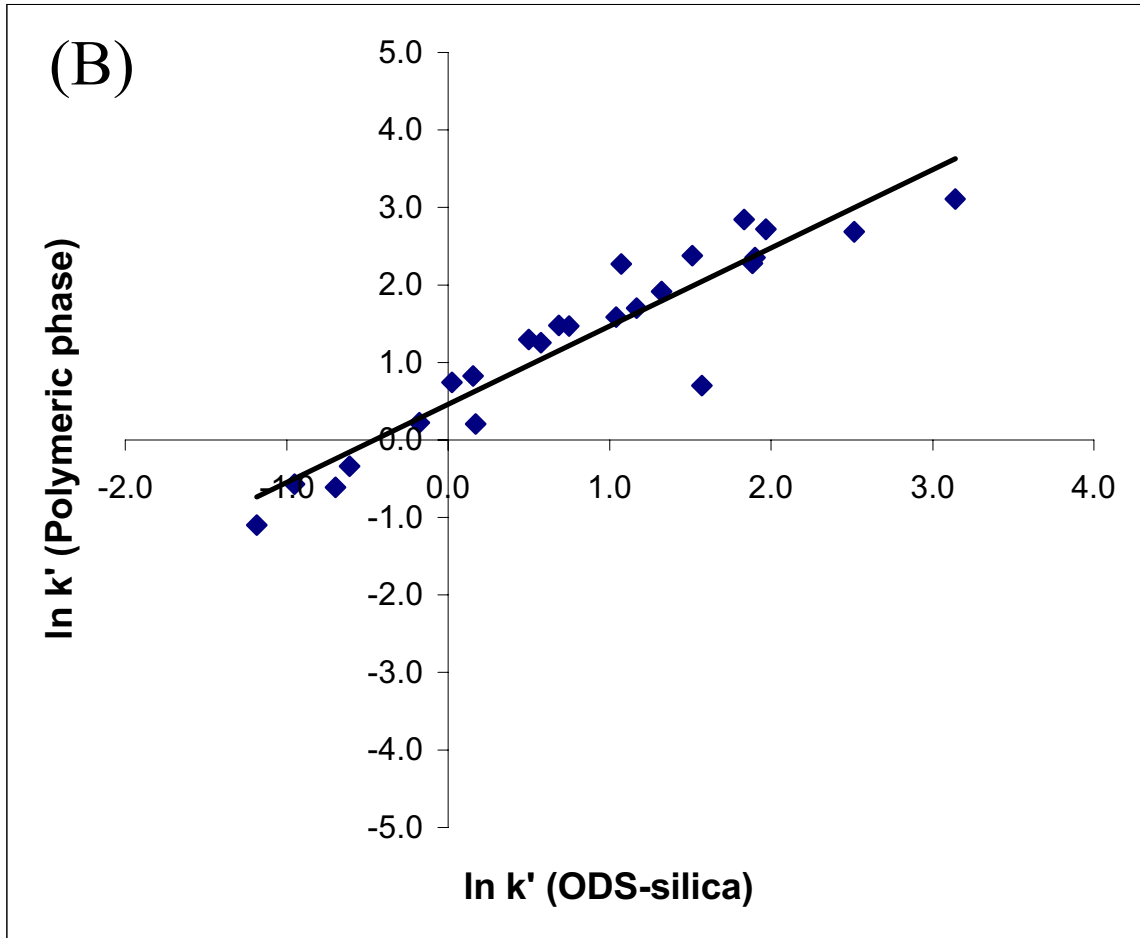
Figure 9A

(A)

ln k' (ZirChrom-PBD) vs ln k' (ODS-silica)
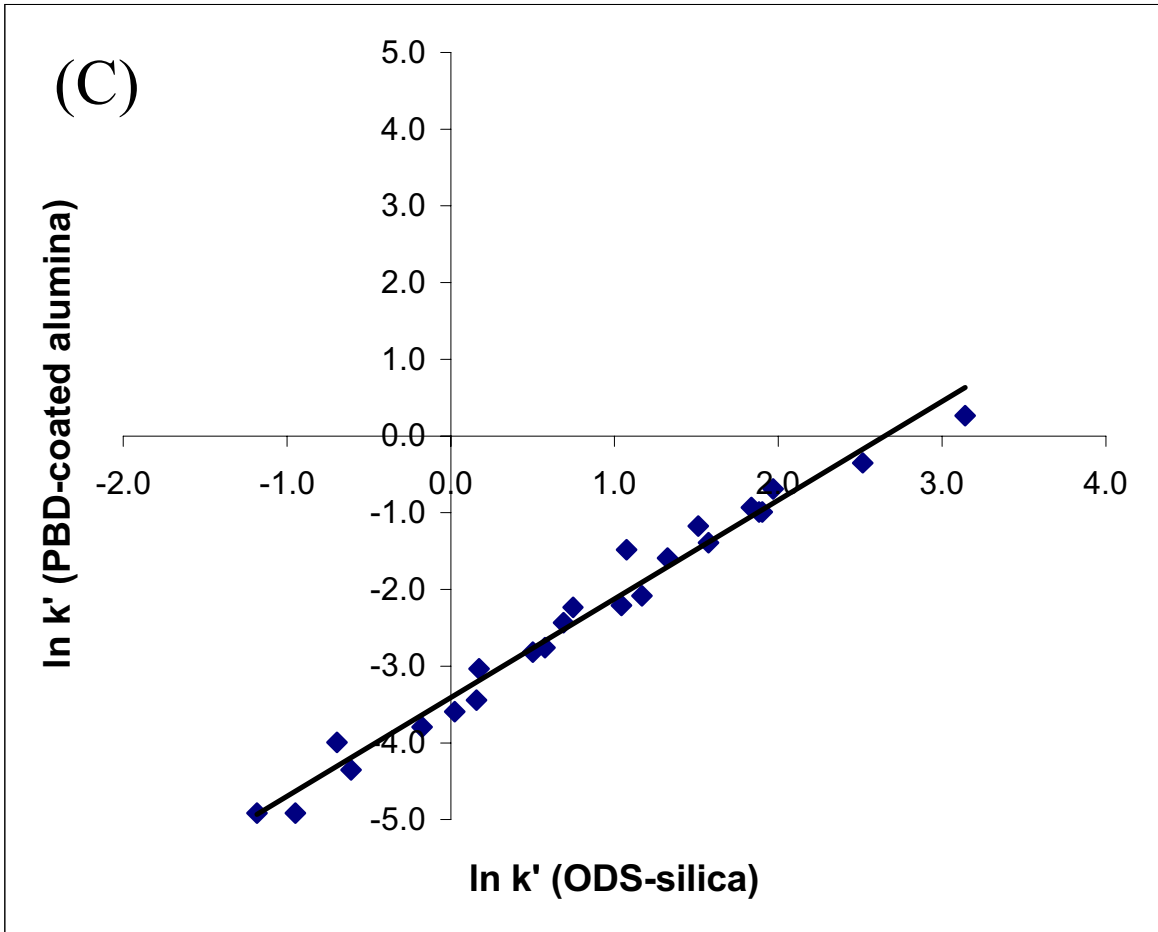
Figure 9B

Figure 9C

Figure 9D
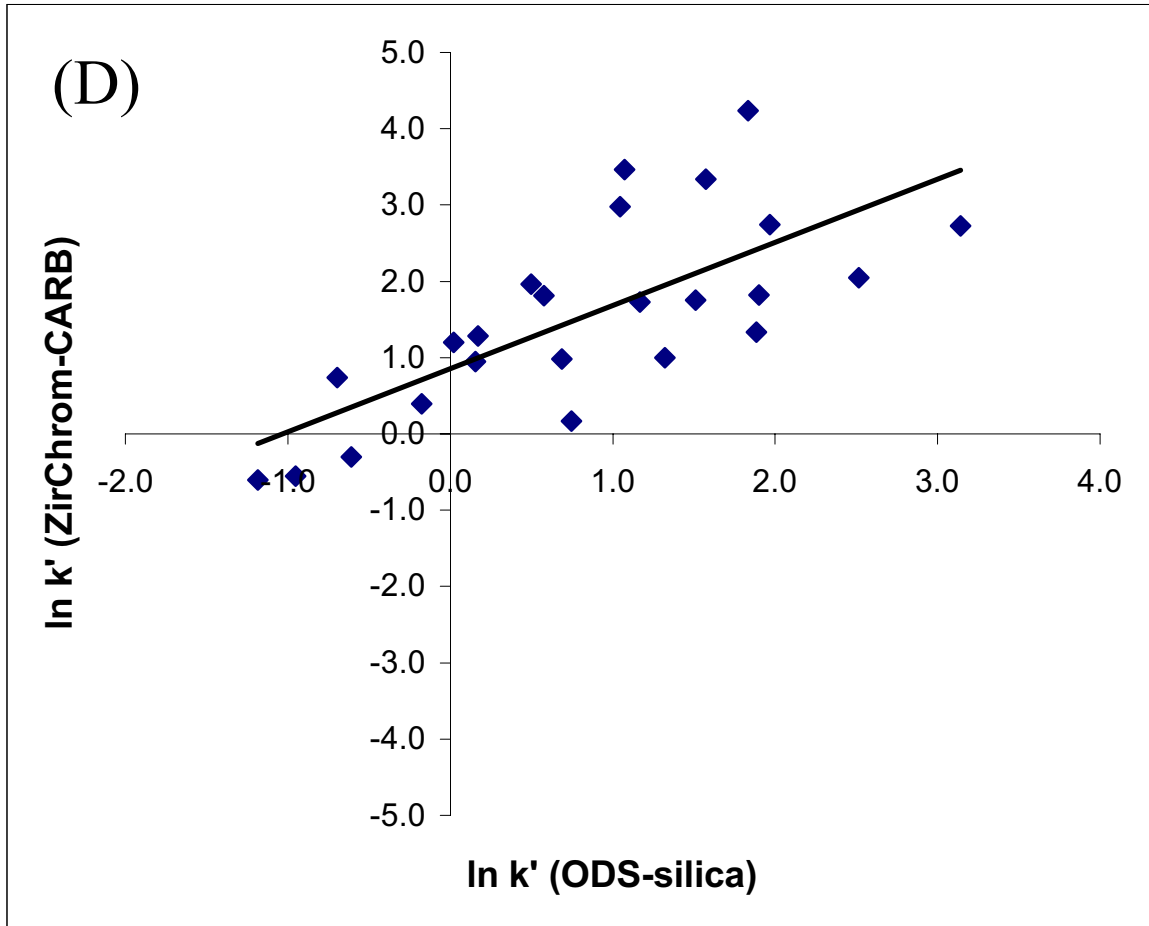
Regression Statistics

Plot	Column	Slope	Intercept	r^2
A	ZirChrom-PBD	1.070 ± 0.0323	-0.954 ± 0.045	0.980
B	Polymeric phase	1.000 ± 0.0816	0.462 ± 0.11	0.874
C	PBD coated alumina	1.290 ± 0.0413	-3.410 ± 0.057	0.978
D	ZirChrom-CARB	0.829 ± 0.176	0.853 ± 0.230	0.529

Figure 10

Figure 11
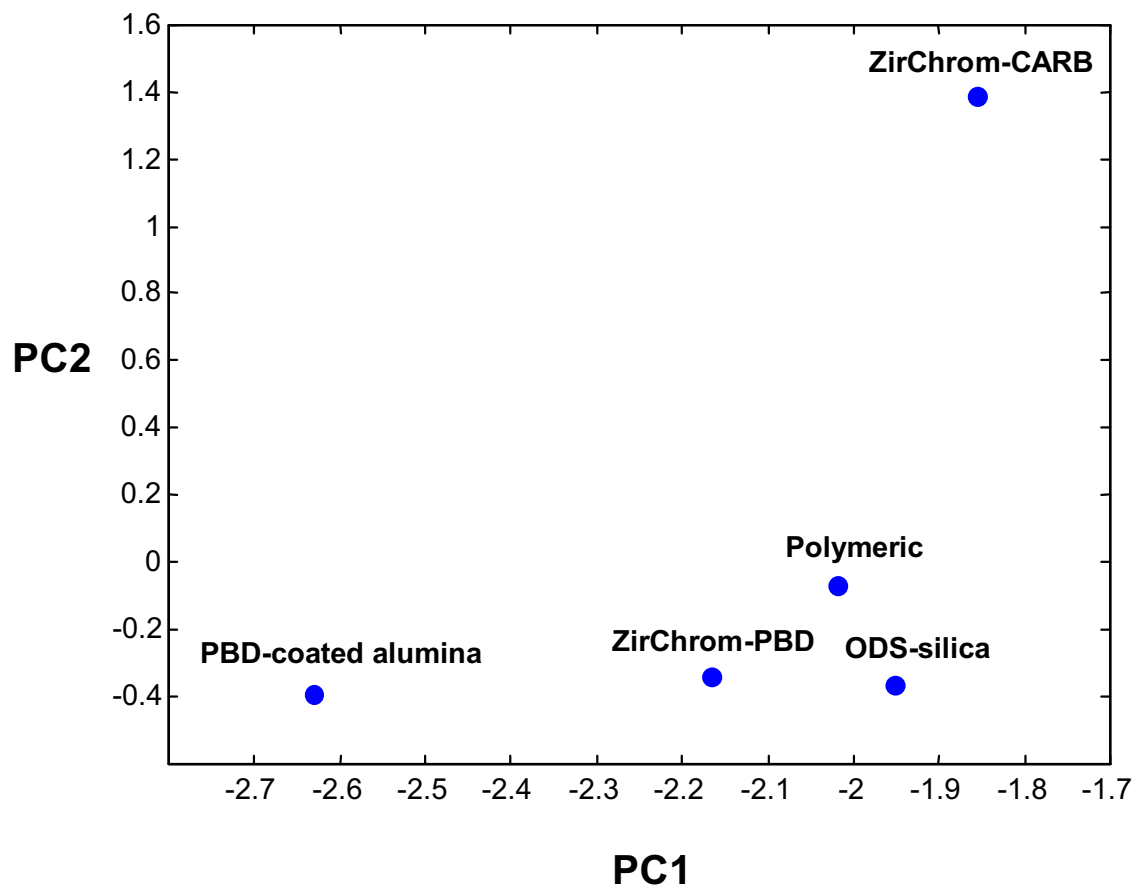
Table I: General Characteristics* of the Five "Stable" RPLC Test Columns

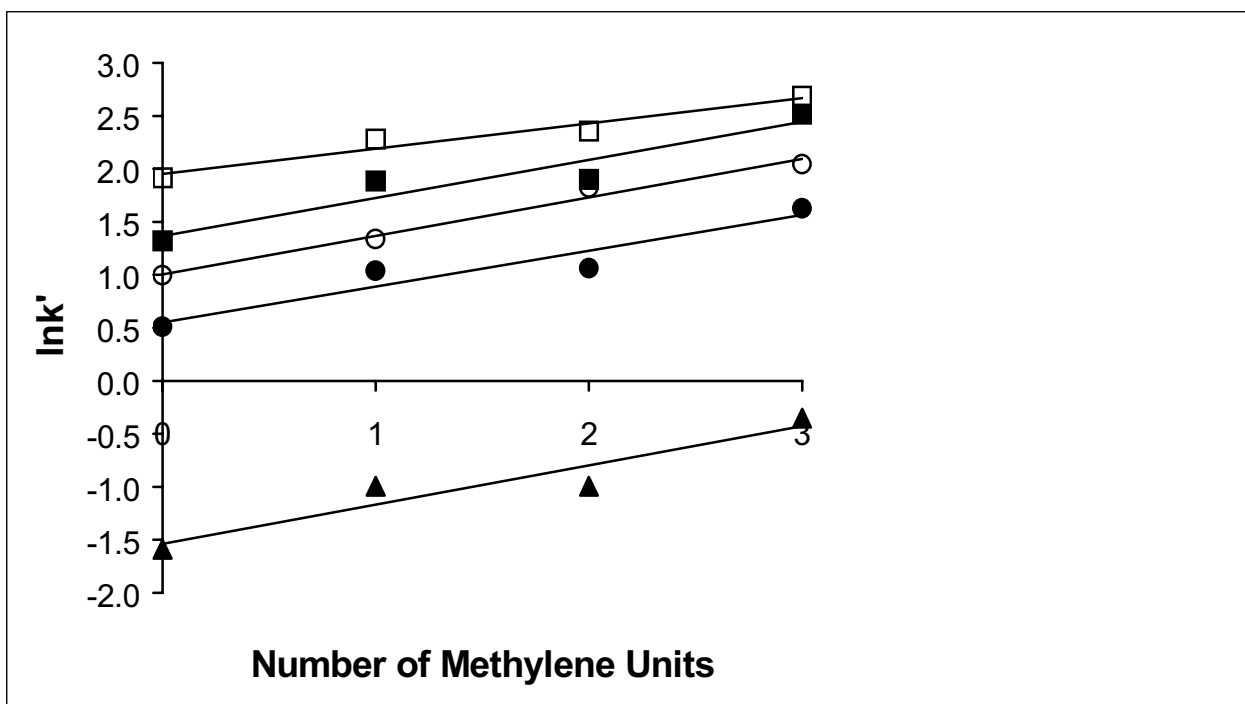| Description	ZirChrom-PBD	ZirChrom-CARB	Polymeric	ODS silica	PBD-coated alumina
Particle Size (μm)	3	3	5	3.5	5
Pore Size (Å)	300	300	100	300	80
Column size (mm)	150 x 4.6	150 x 4.6	150 x 4.6	150 x 4.6	150 x 4.6
Price (US $)	595	695	480	425	695
Low pH Limit	0.5	0.5	1	1.8	1.3
High pH Limit	14	14	14	8	12
Temp Limit (°C)	200	200	150	80	†
Carbon Loading (%C)	3.0	1.1	†	2.8	2.0

* Specifications as reported by manufacturer at the time the study was performed.

† Data not available.

Table II: Reduced Plate Height for 27 Analytes on the Five Test Columns

Analyte	Solute	ZirChrom-PBD	ZirChrom-CARB	ODS Silica	Polymeric	PBD-coated alumina
1	uracil	7.9	12	7.2	9.3	5.7
2	benzyl formamide	7.3	11	6.0	8.2	5.7
3	benzyl alcohol	6.1	7.0	5.6	8.7	5.6
4	phenol	6.0	8.9	5.5	8.1	6.7
5	benzoic acid	8.0	23	6.5	8.8	7.3
6	3-phenyl propanol	7.8	8.1	5.9	23	8.7
7	acetophenone	5.5	9.0	4.8	19	5.9
8	benzonitrile	5.3	12	5.4	15	7.0
9	p-chlorophenol	6.3	17	5.7	11	6.9
10	methyl benzoate	5.5	16	4.7	27	6.4
11	nitrobenzene	4.9	17	4.6	17	5.7
12	anisole	4.7	7.9	4.5	21	7.2
13	benzene	4.6	6.5	4.3	23	6.5
14	pyridine	5.0	107	11	9.3	4.6
15	p-nitrotoluene	4.7	19	4.5	25	5.9
16	n,n-dimethylaniline	5.4	10	8.3	57	5.2
17	p-nitrobenzyl chloride	4.8	9.4	4.5	23	6.2
18	toluene	4.5	7.7	4.3	26	7.2
19	4-butyl benzoic acid	8.3	24	5.4	16	7.4
20	benzophenone	5.3	9.5	4.4	76	6.6
21	bromobenzene	4.1	19	4.6	25	5.5
22	ethyl benzene	4.2	10	4.6	27	7.2
23	p-xylene	4.1	5.6	4.9	33	5.6
24	naphthalene	4.5	92	4.7	68	6.4
25	p-dichlorobenzene	4.0	17	5.0	46	5.8
26	propyl benzene	5.0	13	5.2	34	10
27	butyl benzene	6.0	23	6.2	35	15
	Mean	**5.5**	**19**	**5.5**	**26**	**6.8**
	Median	**5.3**	**12**	**5.0**	**23**	**6.4**
	Lowest*	**4.0**	**5.6**	**4.3**	**8.1**	**4.6**
	Highest†	**8.3**	**107**	**11**	**76**	**15**

*Numbers in parentheses denote analyte with lowest plate count.

† Numbers in parentheses denote analyte with highest plate count.

Table III: Results of Principal Components Analysis of Retention Factor Data

Number of Principal Components	Eigenvalue	Variance (%)	Total Variance (%)	Residual Standard Deviation
1	22.960	89.929	89.929	0.175
2	2.341	9.168	99.096	0.061
3	0.157	0.615	99.711	0.042
4	0.063	0.246	99.957	0.023
5	0.011	0.043	100.000	0.000