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EVALUATION OF NEW HYBRID STATIONARY PHASES WITH COMPREHENSIVE STANDARDIZED CHROMATOGRAPHIC TEST METHODS

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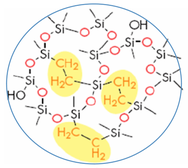
OVERVIEW

The first commercially available hybrid organic/inorganic packing materials were introduced 6 years ago as the XTerra® family of C_{18}, C_8, polar embedded group C_{18} and polar embedded group C_8 stationary phases. A substantially improved brand of reversed-phase hybrid columns (XBridge™ columns) will be commercially available this year. The particles for the new hybrid brand contain bridged ethyl groups (see Fig. 1) in place of the methyl groups of the first generation hybrid.

The development of new packing materials requires the use of standardized chromatographic tests in order to assess the performance of the new packings. Data from our standard tests include: van Deemter experiments; selectivity and peak shape at low and intermediate pH; and stability at high and low pH. Development of a new high-pH stability test—using NaOH at pH 12.3—was required due to the dramatically improved high-pH stability conferred by the bridged ethyl hybrid (BEH) particle and its improved bonding technology.

Unlike commonly used bonding approaches, our improved bonding technology confers excellent stability at low *and* high pH. In methods development, pH has long been recognized as a major parameter influencing selectivity. The presented data supports the use of several BEH stationary phases across an expanded pH range and illustrates the selectivity differences across the various stationary phases.

BEH Technology™



BEH Technology™

U.S. Patent 6,686,035 B2

Figure 1: BEH particles are prepared from two high purity monomers: one that forms SiO_2 inorganic-units and another that forms $O_{1.5}Si\text{-}CH_2CH_2\text{-}SiO_{1.5}$ organosiloxane-units.

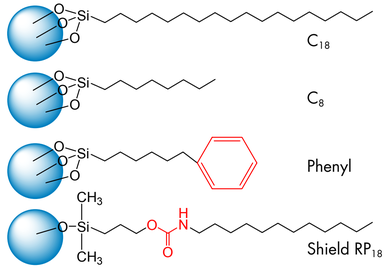


Figure 2: XBridge™ particles are surface bonded with a variety of ligands: trifunctional C_{18}, trifunctional C_8, Shield RP_{18}, and a trifunctional Phenyl.

ACCELERATED HIGH-pH AGING

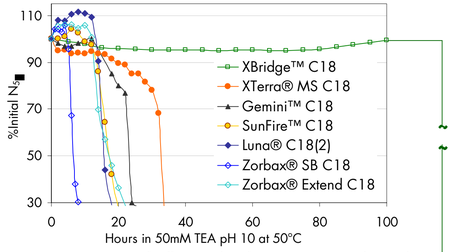


Figure 3: Comparison of efficiency loss for C_{18} stationary phases during exposure to **50mM TEA pH 10** mobile phase at 50°C. Analyte: Acenaphthene. Test mobile phase: 65/35 methanol /20mM K_2HPO_4, pH 7.0 (v/v) at 50°C. Test is described in detail elsewhere[1].

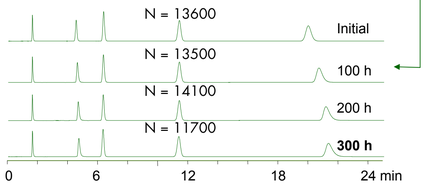


Figure 4: XBridge™ C_{18} after 0, 100, 200, and 300 hours of exposure to **50mM TEA pH 10** mobile phase at 50°C. Analytes: (1) Uracil, (2) Propranolol, (3) Naphthalene, (4) Acenaphthene, and (5) Amitriptyline. Test mobile phase: 65/35 methanol /20mM K_2HPO_4, pH 7.0 (v/v) at 50°C.

Figure 5: Comparison of efficiency and retention stability for the XBridge™ and benchmark columns at pH 12.3. Results reported in hours of exposure to **0.02N NaOH pH 12.3** mobile phase at 50°C until (A) 50% efficiency loss and (B) 25% retention factor loss. Analyte: Decanophenone. Test mobile phase: 50/50 acetonitrile/water (v/v).

ACCELERATED LOW-pH AGING

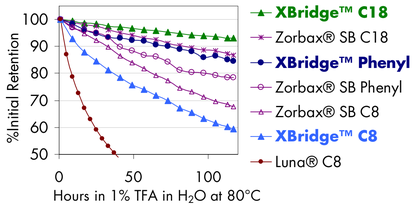


Figure 6: The **1% trifluoroacetic acid (TFA) pH 1.0 stability** of the XBridge™ trifunctional stationary phases compared to several benchmark columns. Analyte: Benzene. Test is described in detail elsewhere[2].

- Although the low pH stability of the XBridge™ C_8 is not as good as that of the XBridge™ C_{18} or Phenyl, its stability is substantially better than most of the leading brands of C_8 stationary phases.
- The XBridge™ C_{18} and Phenyl stationary phases have the best low pH stability of any of the respective class of tested phases.

EFFICIENCY AND PEAK SHAPE

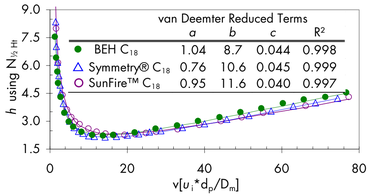


Figure 7: Comparison of reduced *a*, *b*, and *c* terms from the van Deemter equation[3] between 5µm silica-based and BEH C_{18} stationary phases. Test mobile phase: 70/30 acetonitrile/water (v/v) at 30°C. Analyte: Decanophenone.

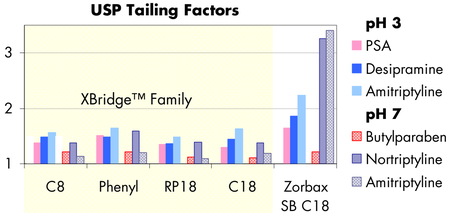


Figure 8: Peak shape comparisons at pH 3 and pH 7 for acids (0.13 µg pyrene sulfonic acid (PSA) and 0.28 µg butylparaben), 2° amines (0.20 µg desipramine and 1.4 µg nortriptyline–injected as a single component), and a 3° amine (amitriptyline, 0.27 µg at pH 3 and 1.4 µg at pH 7).

SELECTIVITY AND PEAK SHAPE

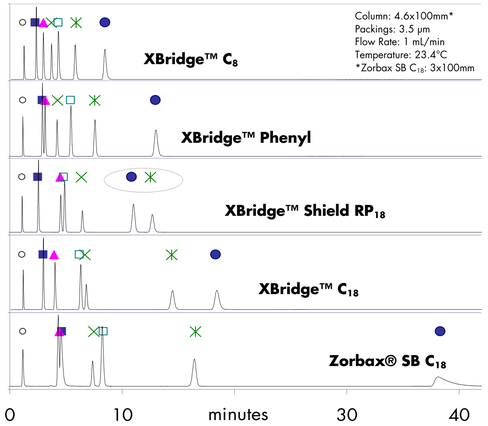


Figure 9: Selectivity and peak shape of XBridge™ stationary phases compared to Zorbax® SB C_{18} in 65/35 methanol /20mM K_2HPO_4, **pH 7.0** (v/v) . Test is described in detail elsewhere[4]. Analytes: ○ Uracil, ■ Propranolol, ▲ Butylparaben, □ Dipropylphthalate, × Naphthalene, ✳ Acenaphthene, and ● Amitriptyline.

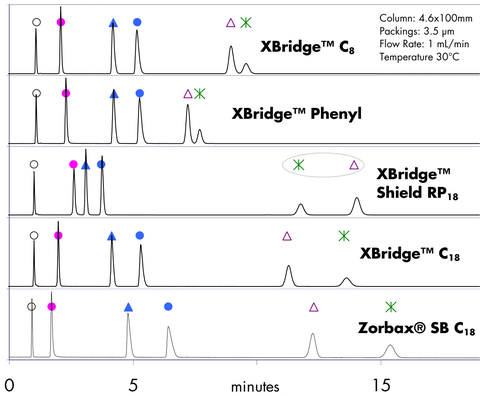


Figure 10: Selectivity and peak shape of XBridge™ stationary phases compared to Zorbax® SB C_{18} in 35/65 acetonitrile/15.4mM $HCOONH_4$, **pH 3.0** (v/v). Analytes: ○ Uracil, ● Pyrene Sulfonic Acid, ▲ Desipramine, ● Amitriptyline, △ Butylparaben, and ✳ Toluene.

CONCLUSIONS

XBridge™ Stability

- The use of trifunctional ligands in combination with our proprietary endcapping technology provides stability at both high *and* low pH (Figures 3, 4, 5 and 6). This improved stability allows the use of broader pH ranges in methods development.
- The most stable stationary phase tested at both low *and* high pH is the XBridge™ C_{18} (Figures 3, 4, 5, and 6).
- The XBridge™ Phenyl is the most stable phenyl stationary phase that we have tested (Figure 5 and Figure 6).
- The XBridge™ C_8 outperforms silica-based C_{18} stationary phases at high pH and is among the most stable C_8 phases we have tested at low pH (Figures 5 and 6).

Selectivity in XBridge™ Family

- The family of XBridge™ stationary phases provides complementary selectivity across the pH ranges.
- At pH 7 (Figure 9) :
  - Peak reversals were observed for amitriptyline and acenaphthene on the Shield RP_{18} phase compared to the other phases. The Shield RP_{18} phase has the *lowest* retention for the bases relative to acenaphthene.
  - The phenyl phase offers the *highest* retentions for basic analytes relative to acenaphthene compared to the other phases. High relative retention for the proton accepting analyte (i.e. dipropylphthalate) was also observed.
  - Differences in hydrophobicity are apparent across the family of stationary phases: C_8 < Phenyl < Shield RP_{18} <C_{18}.
- At pH 3 (Figure 10):
  - Peak reversals were observed for butylparaben and toluene on the Shield RP_{18} phase compared to the other phases.
  - Acids and proton donating (i.e. butylparaben) analytes were most strongly retained on the Shield RP_{18} phase. However, the retention for PSA relative to toluene was *highest* on the Phenyl phase.
  - The hydrophobicity ranking of the phase was different from that observed at pH 7: Phenyl < C_8 < Shield RP_{18} <C_{18}. This effect was found to be due to the change from methanol to acetonitrile as the mobile phase modifier. This makes the retention of the ionized analytes relative to toluene highest on the Phenyl phase.

Peak Shape and Efficiency Summary

The family of XBridge™ stationary phases provides excellent efficiency and peak shape at pH 3 and pH 7.

- The van Deemter data (Figure 7) illustrate the comparable performance of BEH C_{18} and silica-based C_{18} phases.
- The XBridge™ tailing factors for acids and bases at both pH 3 and pH 7 are <1.7, providing excellent peak shape (Figure 8) for optimum resolution.

References

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