Supporting Information for

Further Evaluation of the Base Stability of Hydrophilic Interaction Chromatography Columns Packed with Silica or Ethylene-Bridged Hybrid Particles

Thomas H. Walter*, Cheryl Boissel, Jessica A. Field and Nicole L. Lawrence

Waters Corporation, 34 Maple Street, Milford, MA

*Corresponding Author

Tel.: (508) 498-9633. Email: tom_walter@waters.com.

Table of Contents

Page S2 - Figure S1: Comparison of relative changes in average tailing factor and efficiency for a BEH130 HILIC Column exposed to 100% aqueous pH 11.30 10 mM AmBic at 70°C

Page S3 - Figure S2: Relative changes in retention factors for BEH130 HILIC Columns exposed at 70°C to basic challenge solutions with different water concentrations

Page S4 - Figure S3: Relative changes in retention factors for columns packed with BEH materials of different surface area exposed at 70°C to 100% aqueous pH 11.30 10 mM AmBic

Page S5 - Figure S4: Relative changes in retention factors for columns packed with BEH Sulfobetaine materials of different surface area exposed at 70°C to 100% aqueous pH 11.30 10 mM AmBic

Page S6 - Figure S5: Relative changes in retention factors for three commercially available HILIC columns exposed at 70°C to 100% aqueous pH 11.30 10 mM AmBic

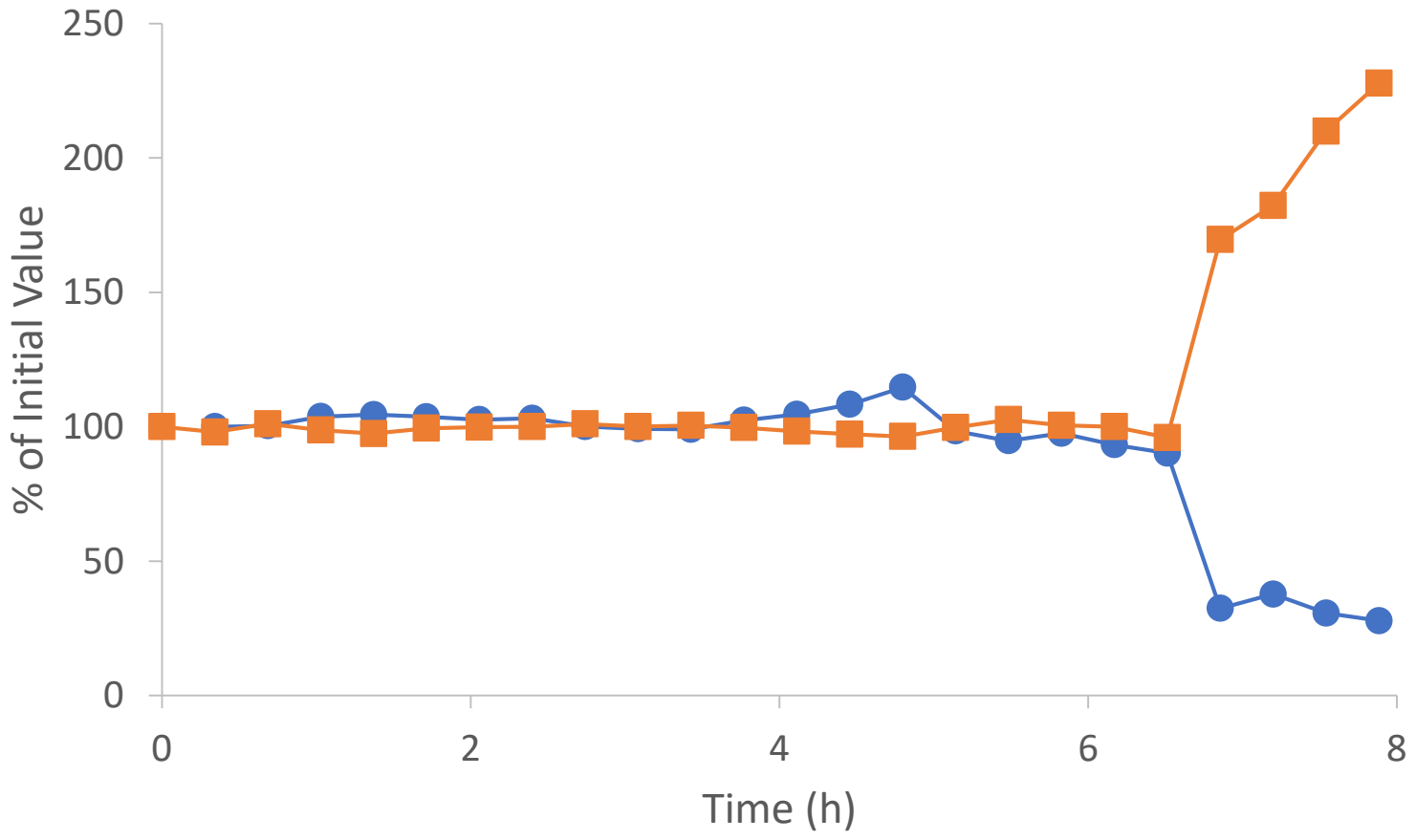
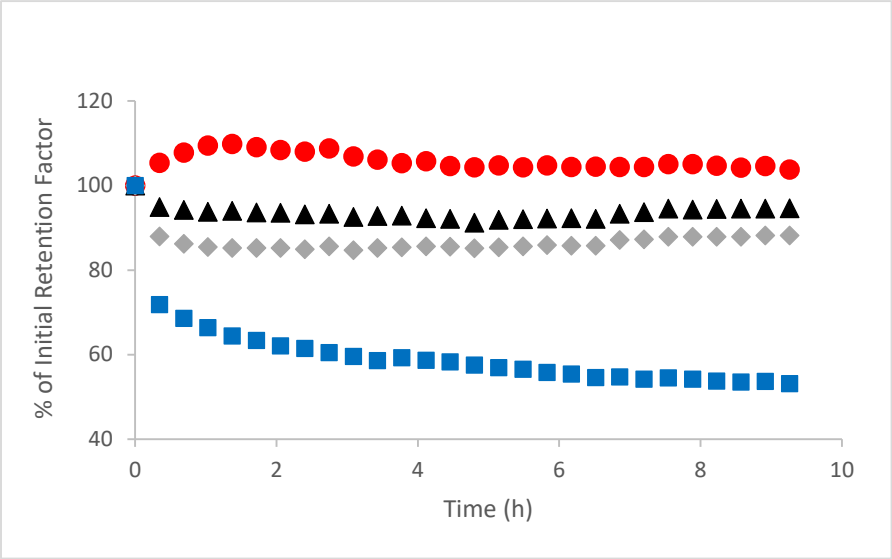
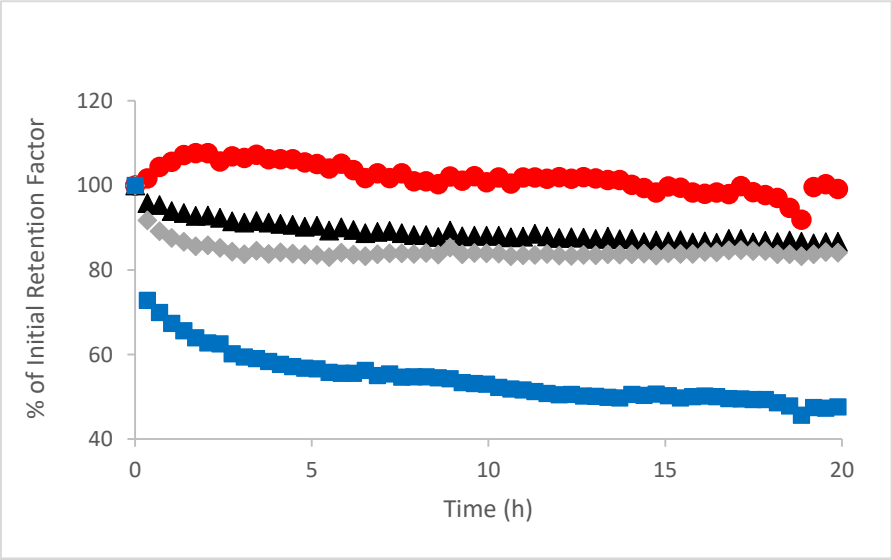


Figure S1: Comparison of the % of the initial average tailing factor and efficiency for a 2.1 x 50 mm 1.7 µm BEH130 HILIC Column *vs* time exposed to 100% pH 11.30 10 mM ammonium bicarbonate (AmBic) at 70°C (blue circles, efficiency; orange squares, tailing factor).

A



B



C

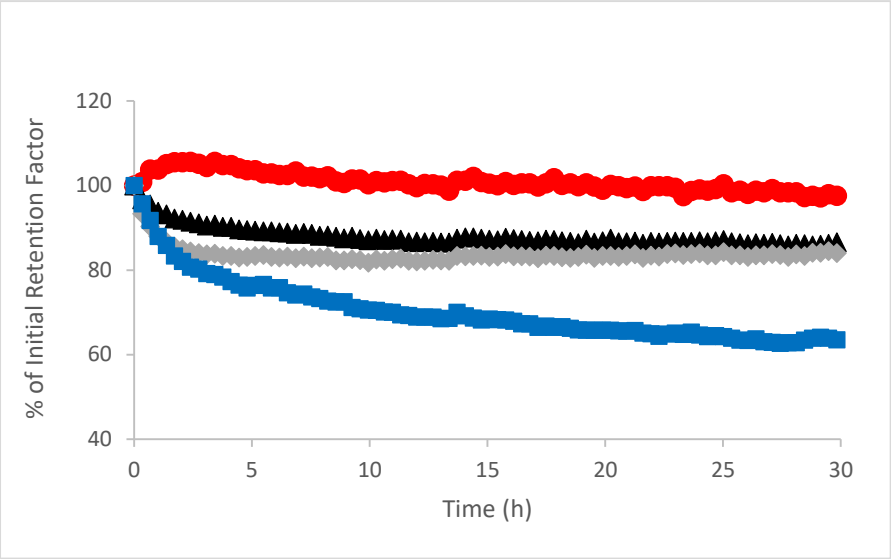
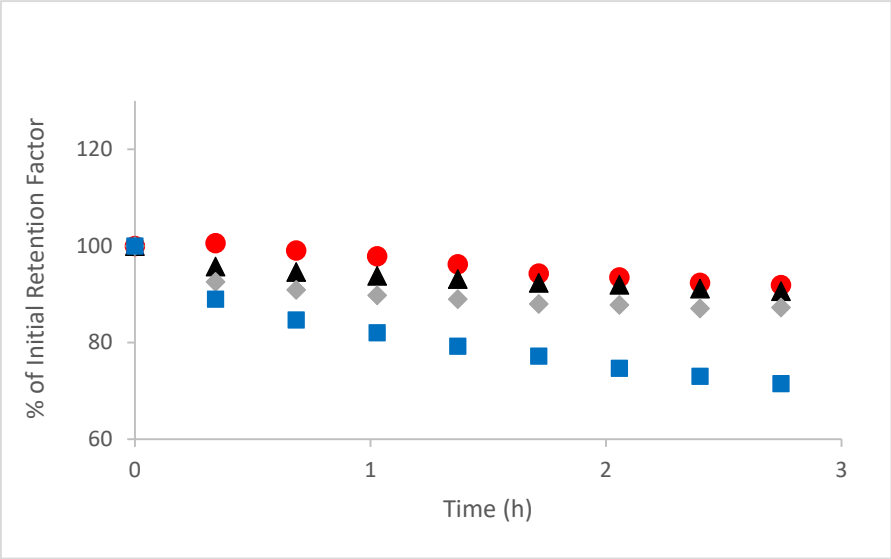
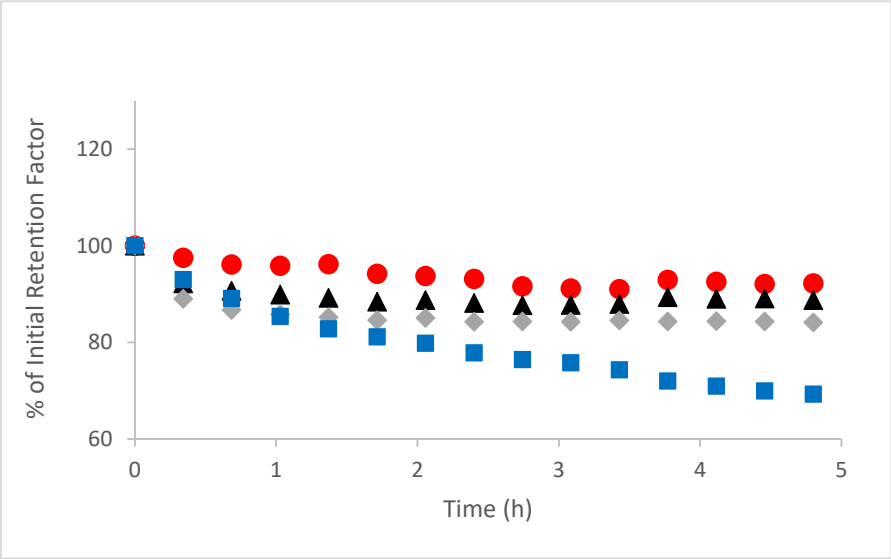


Figure S2: Relative changes in retention factors during base stability tests of 2.1 x 50 mm 1.7 µm BEH130 HILIC Columns, shown as % of initial retention factor *vs* time exposed to the basic solution (blue squares – TMPA, black triangles – cytosine, gray diamonds - adenine, red circles - TS). The columns were exposed at 70°C to solutions containing (A) 100% pH 11.30 10 mM AmBic, (B) 10/90 v/v acetonitrile/$^{w}_{w}$pH 11.30 10 mM AmBic, (C) 20/80 acetonitrile/$^{w}_{w}$pH 11.30 10 mM AmBic.

A



B



C

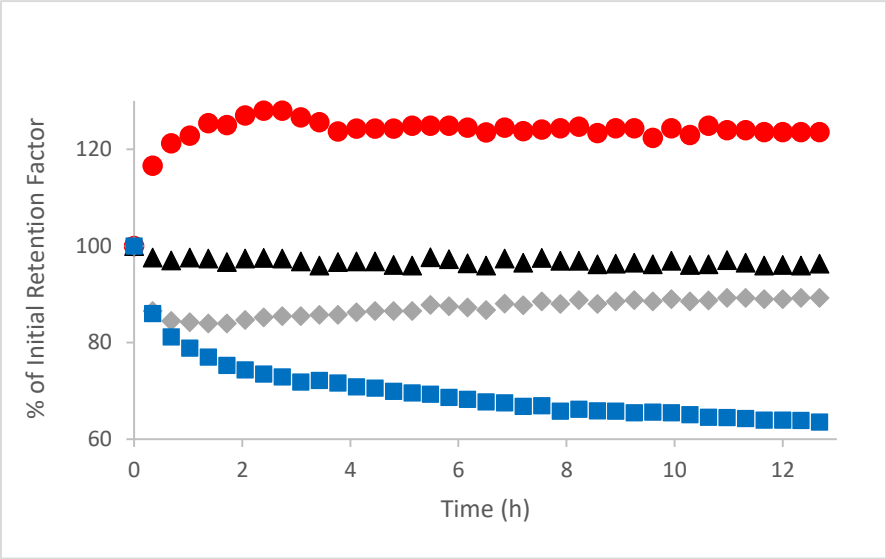
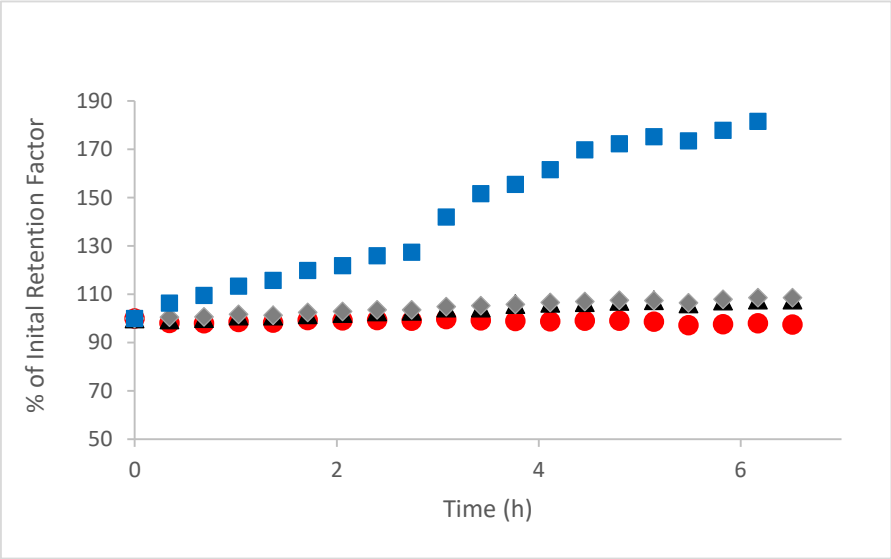


Figure S3: Relative changes in retention factors during base stability tests of 2.1 x 50 mm columns packed with 1.7 µm unbonded BEH materials of varying surface area, shown as % of initial retention factor *vs* time exposed to the basic solution (blue squares – TMPA, black triangles – cytosine, gray diamonds - adenine, red circles - TS). The columns were exposed at 70°C to a solution containing 100% pH 11.30 10 mM AmBic: (A) BEH65 (399 m^2/g), (B) BEH95 (273 m^2/g), (C) BEH300 (92 m^2/g).

A



B

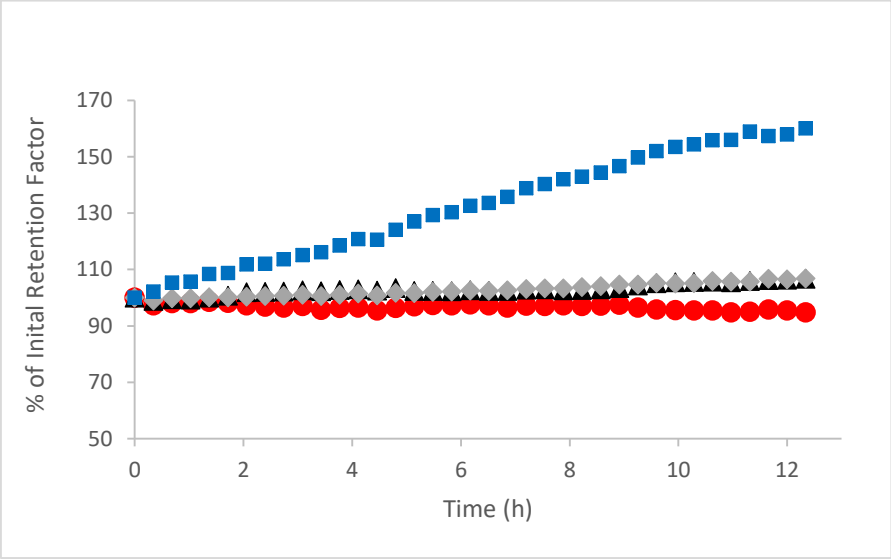
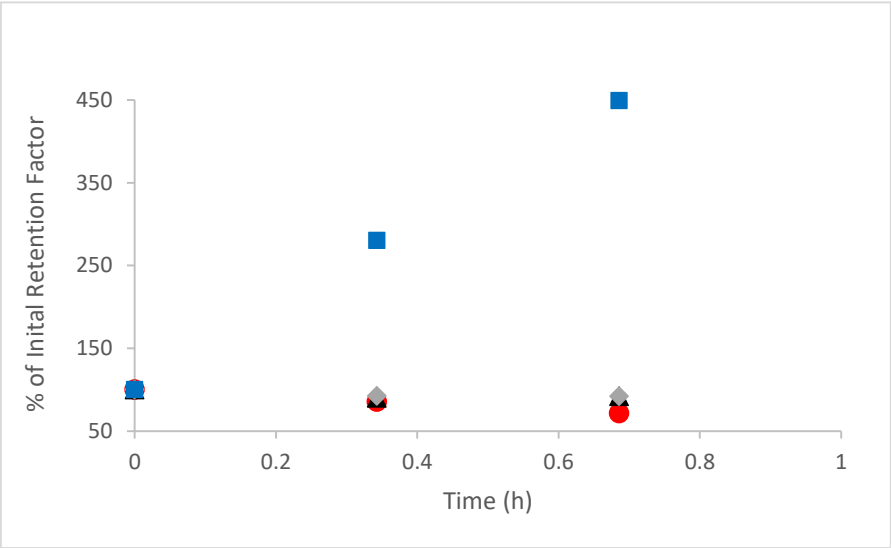
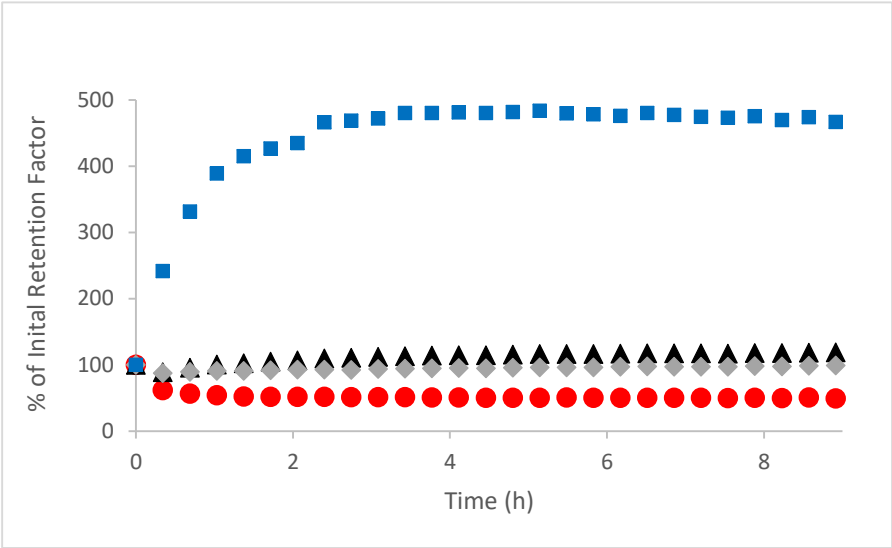


Figure S4: Relative changes in retention factors during base stability tests of 2.1 x 50 mm columns packed with 1.7 µm BEH Sulfobetaine materials of varying surface area, shown as % of initial retention factor *vs* time exposed to the basic solution (blue squares – TMPA, black triangles – cytosine, gray diamonds - adenine, red circles - TS). The columns were exposed at 70°C to a solution containing 100% pH 11.30 10 mM AmBic: (A) BEH95 S (172 m^2/g), (B) BEH130 S (133 m^2/g)

A



C



B

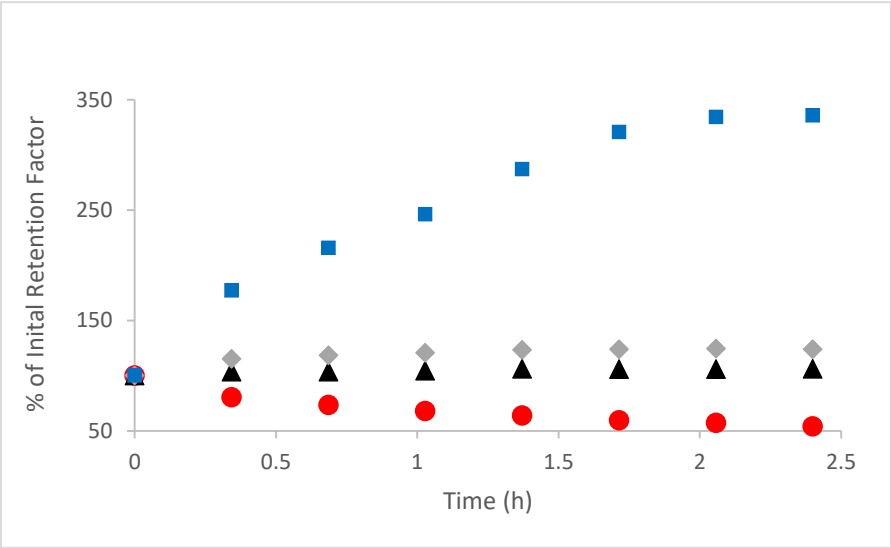


Figure S5: Relative changes in retention factors during base stability tests of several HILIC columns, shown as % of initial retention factor *vs* time exposed to the basic solution (blue squares – TMPA, black triangles – cytosine, gray diamonds - adenine, red circles - TS). The columns were exposed at 70°C to a solution containing 100% pH 11.30 10 mM AmBic: (A) Luna NH2 Column, (B) Poroshell 120 HILIC-Z Column, (C) Torus Diol Column.