Evaluation of the Stability of New Organic/Inorganic Hybrid Reversed-Phase HPLC Packings

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Abstract:

We have recently developed a new family of reversed-phase HPLC packings based on organic/inorganic hybrid particles. To evaluate the hydrolytic stability of these packings relative to state-of-the-art C_{18}-silicas, we have used several different test protocols. These protocols include chromatographic tests using mobile phases containing buffers that range from pH 1.2 to 11.5, as well as temperatures up to 60 °C. The results show that the packings based on hybrid particles exhibit exceptional stability in alkaline mobile phases and excellent stability in acidic mobile phases.

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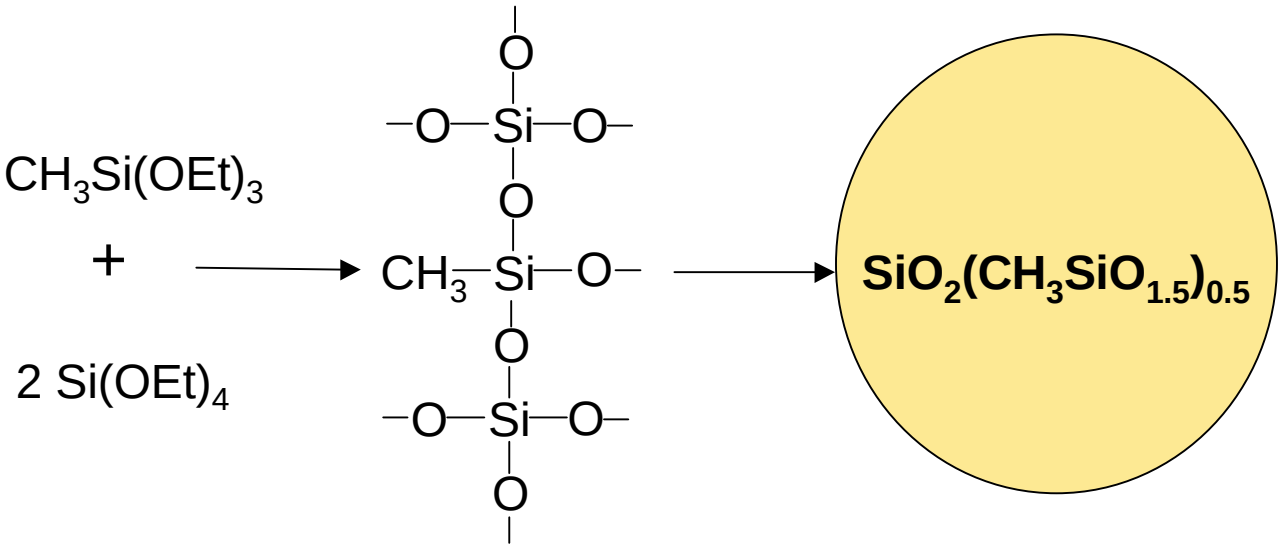
Hybrid Technology Particle

- Hybrid Organic/Inorganic materials contain both organic and inorganic components

- The hybrid particles described here were synthesized from $Si(OEt)_4$ and $CH_3Si(OEt)_3$:



- Hybrid particles combine:
  - efficiency and mechanical strength of silica
  - extended pH range and absence of base tailing of organic polymers

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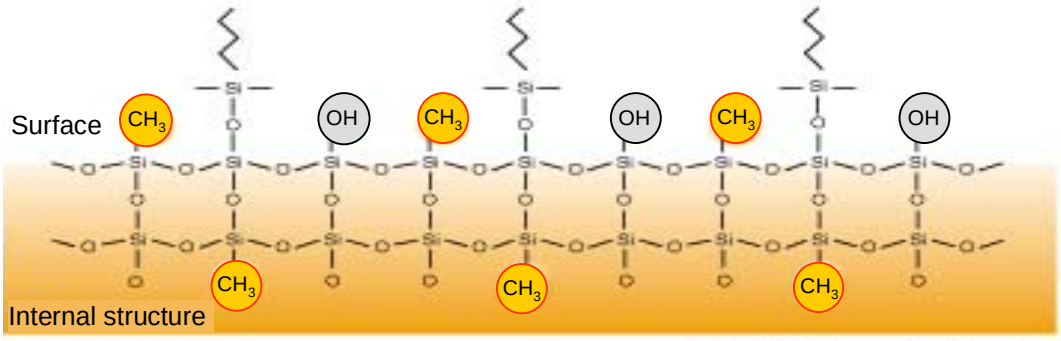
XTerra™ Bonded Phases



Bonded XTerra™ Particle

- XTerra™ bonded phases are based on the hybrid particles shown in the previous slide
- XTerra™ RP_{18}
  - Embedded carbamate C_{18} phase with a trimethylsilyl endcap
  - 14.95% C
  - 2.32 µmol/m²
- XTerra™ MS C_{18}
  - Trifunctional C_{18} phase with a trimethylsilyl endcap
  - 15.45% C
  - 2.22 µmol/m²

pH 10 Et_3N/ 50 °C Accelerated Test Protocol

Accelerated Test Procedure:

1. **Equilibrate**: 45 min, 1 mL/min, test mobile phase
2. **Test**: Mobile Phase: pH 7.0 K_2HPO_4 / MeOH 35:65 v/v, Flow Rate: 1 mL/min, Detection wavelength 254 nm
3. **Challenge**: 50 mM $N(CH_2CH_3)_3 \cdot HCl$ buffer pH 10.0

   Time: 60 min, Flow Rate: 2 mL/min
4. **Purge**: 10 min at 2 mL/min with water

   10 min at 2 mL/min with methanol
5. **Cycle** back to step 1 and repeat until column fails

The column (4.6x150mm) is maintained at 50°C throughout the entire test.

– Under these aggressive conditions XTerra™ columns lasted significantly longer than state-of-the-art C_{18}-silica columns.

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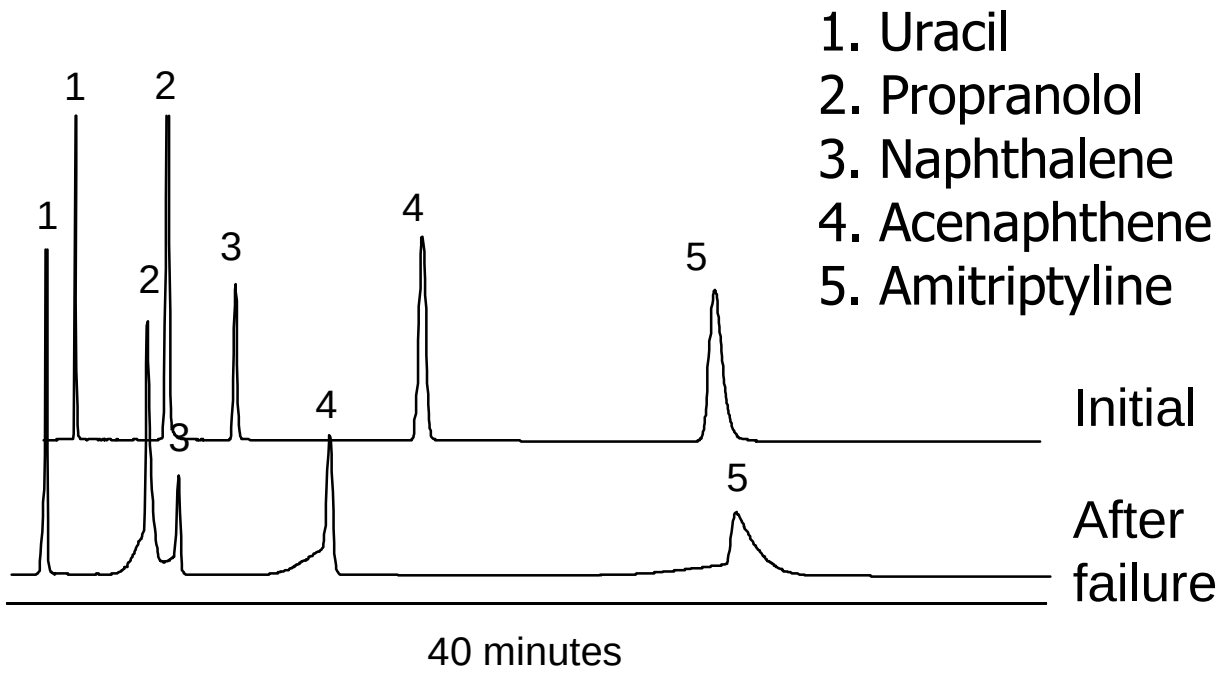
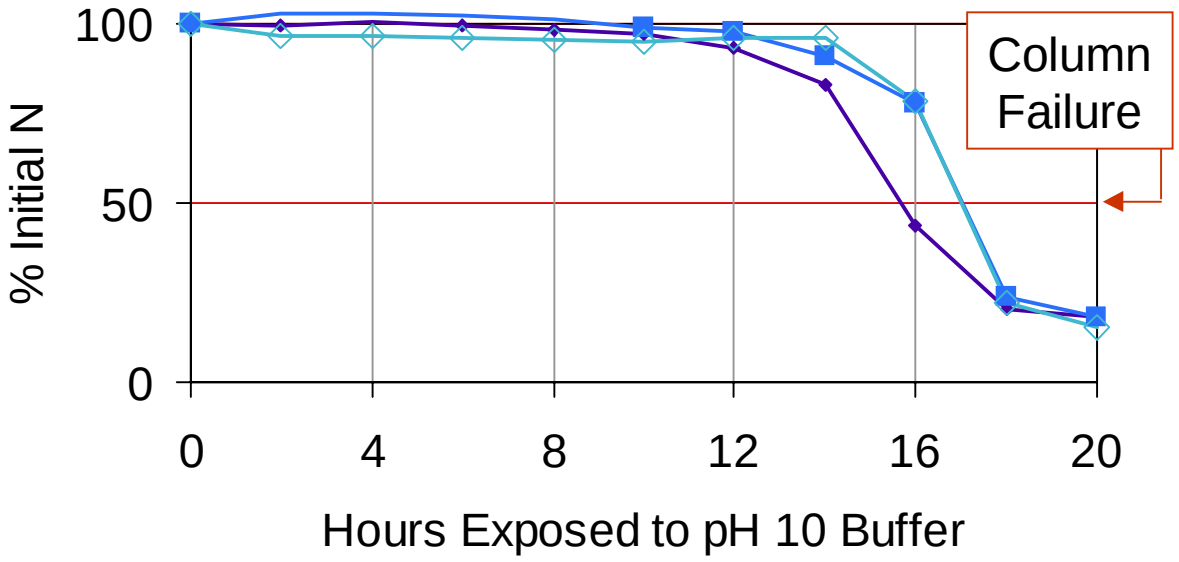
pH 10 Et_3N/ 50 °C Accelerated Test Chromatograms

Typical Chromatograms for C_{18}-Silica S

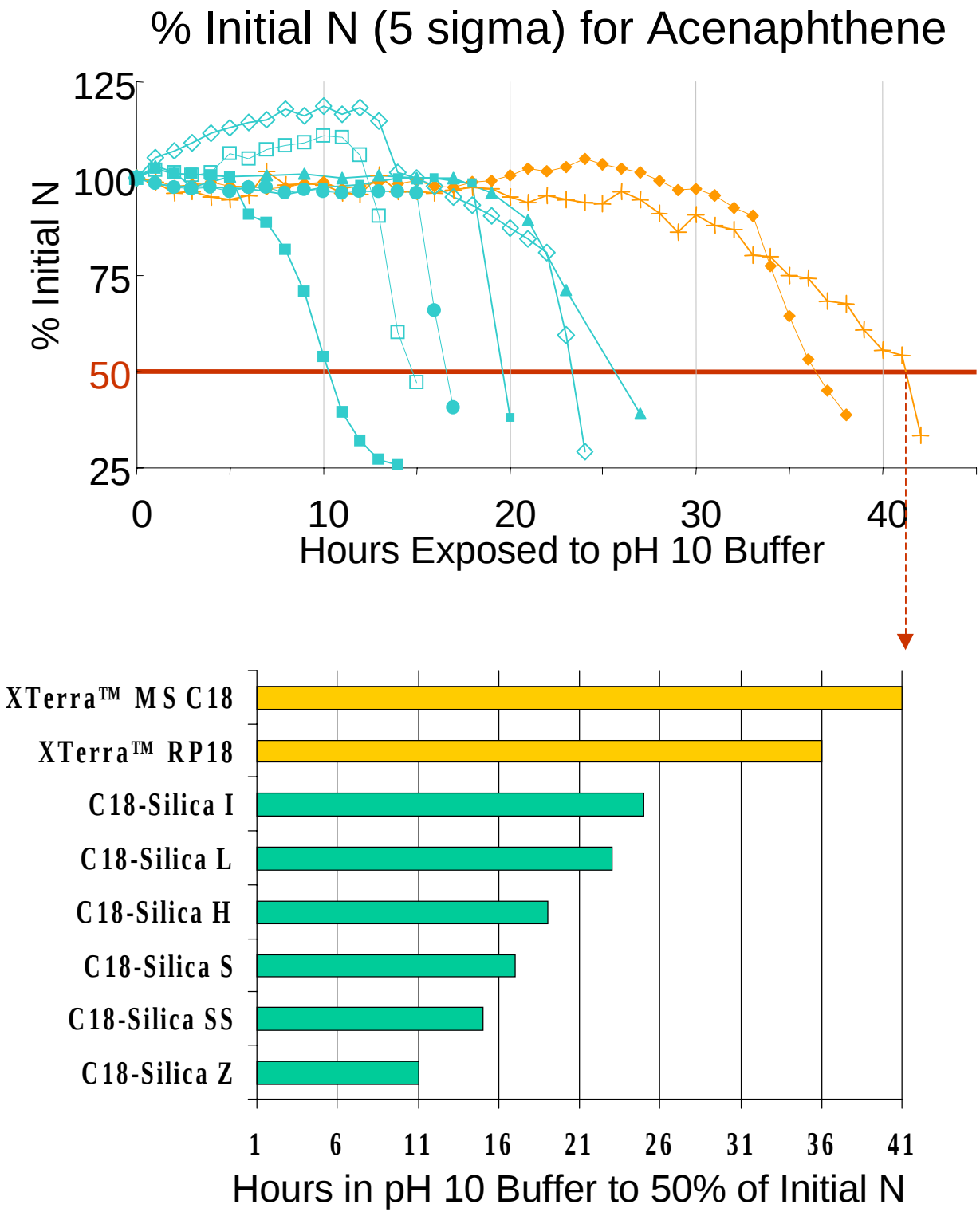


Typical Results - % of Initial Efficiency for Acenaphthene Reproducibility for 3 C_{18}-Silica S Columns



XTerra COLUMNS

pH 10 Et_3N/ 50 °C Accelerated Test Efficiency *vs.* Time Curves



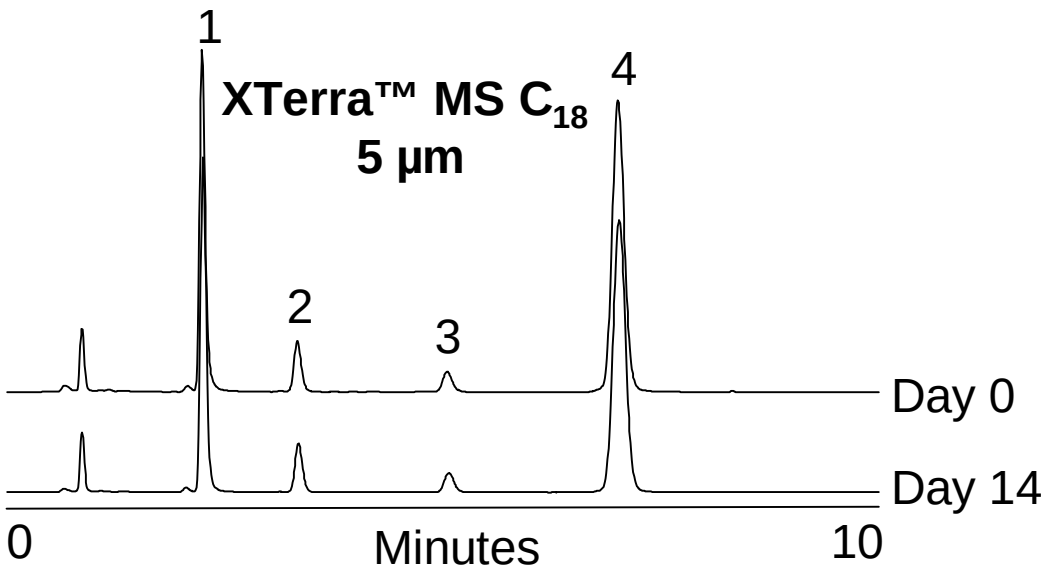
pH 7 Na_2HPO_4/60 °C Accelerated Test Protocol

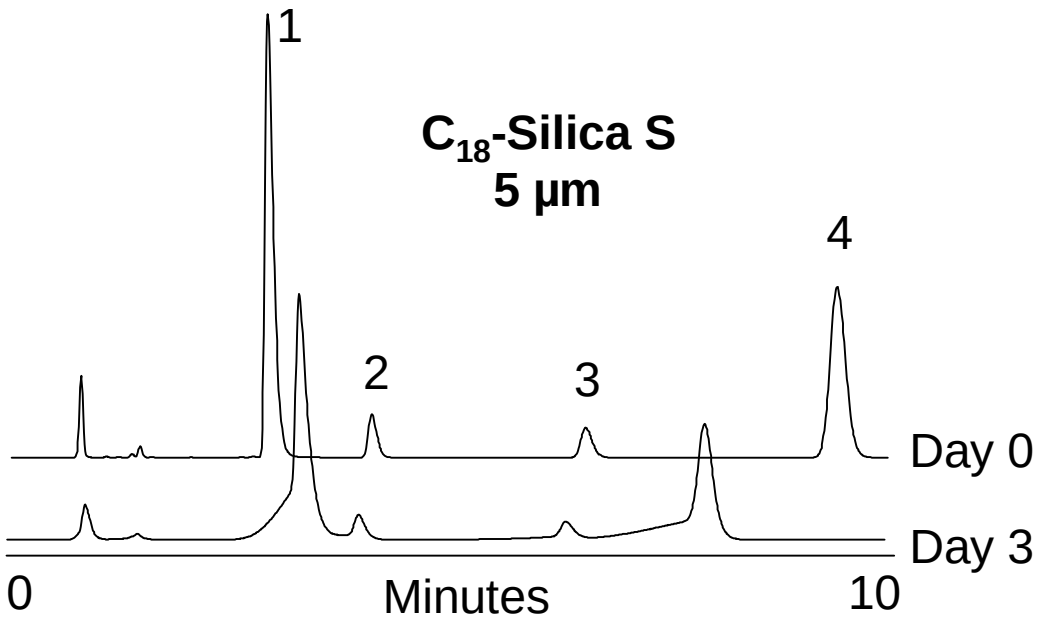
Accelerated Test Procedure:

1. **Equilibrate:** 60 min, 1.0 mL/min, test mobile phase 1
2. **Test Probe Mobile Phase1:** MeCN/H_2O (percentages vary with packing material), Flow rate: 1.0 mL/min, Detection wavelength: 254 nm, probes: uracil, acenaphthene
3. **Equilibrate:** 60 min, 1.5 mL/min, test mobile phase 2
4. **Test Probe Mobile Phase2:** 10 mM pH 7 Na_2HPO_4/MeCN (percentages vary with packing material), Flow rate: 1.5 mL/min, Detection wavelength: 254 nm, probes: tricyclic antidepressants
5. **Purge:** 20 min at 1 mL/min with water
   10 min at 1 mL/min with acetonitrile
   20 min at 1 mL/min with H_2O/MeCN, 90:10
6. **Column Aging Mobile Phase:** 50 mM pH 7 Na_2HPO_4/MeCN 80:20, Flow Rate: 1.0 mL/min
   Time: 60, 120, 180, 240, 240... min
7. **Purge:** 20 min at 1 mL/min with water
   10 min at 1 mL/min with acetonitrile
8. **Cycle** back to step 1 and repeat until column fails.

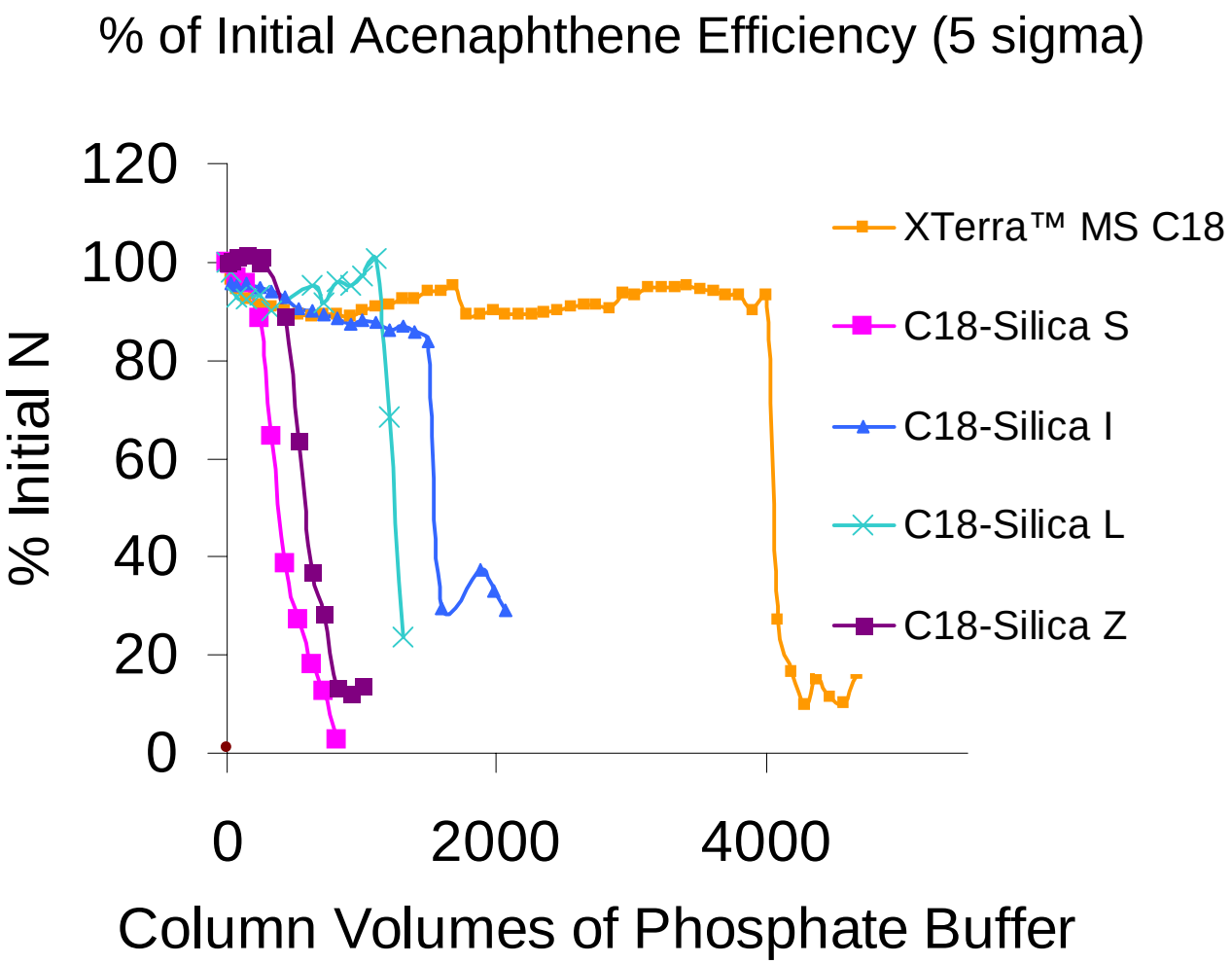
The column (4.6x150mm) is maintained at 60°C throughout the entire test.

pH 7 Na_2HPO_4/60 °C Accelerated Test Chromatograms





pH 7 Na_2HPO_4/60 °C Accelerated Test Efficiency *vs.* Time Curves



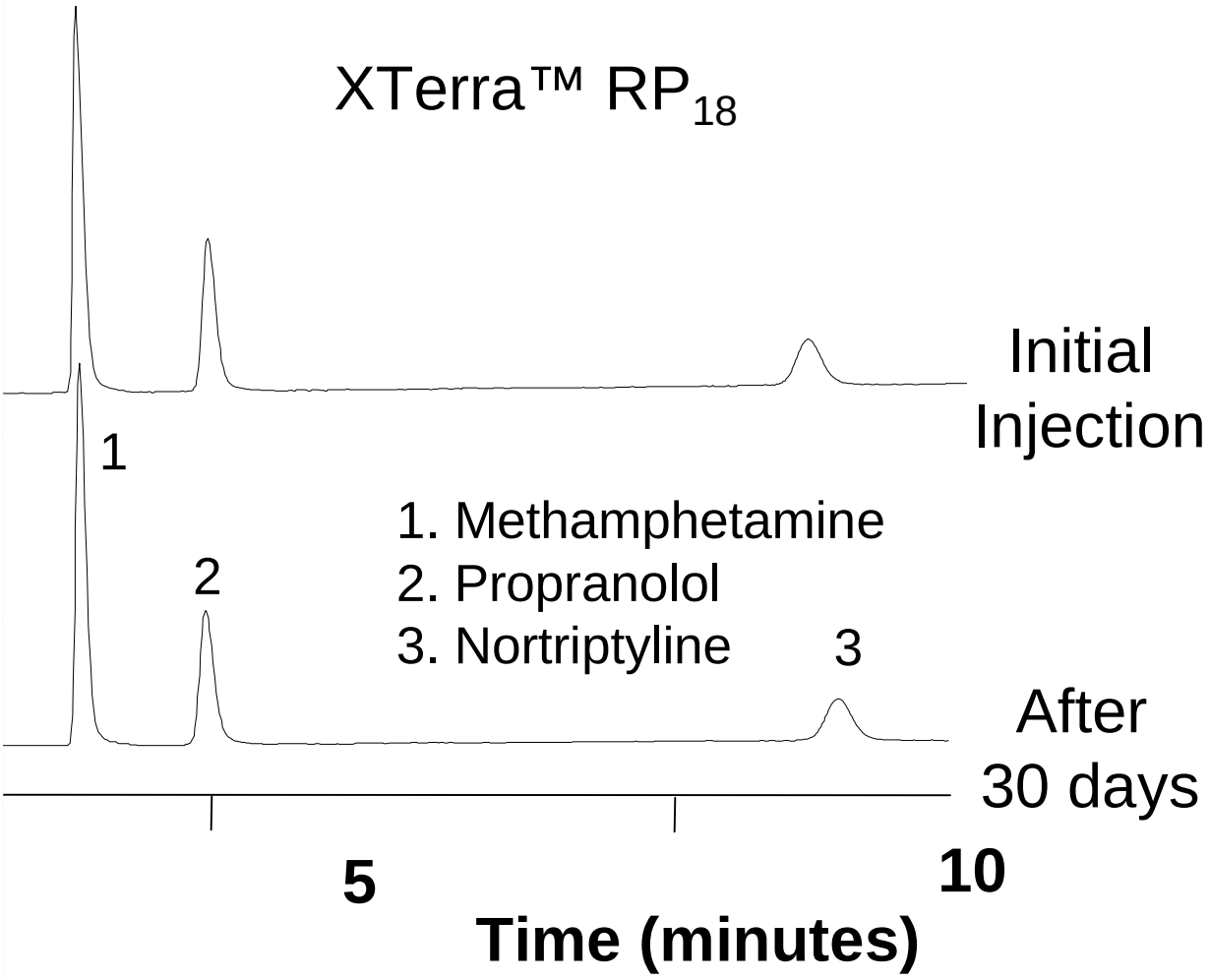
– Using aggressive phosphate buffers at high temperature, bonded hybrid packings resisted dissolution which leads to the premature failure of C_{18}-silica columns.

pH 11.5 Lifetime Test Protocol

pH 11.5 Lifetime HPLC Conditions:

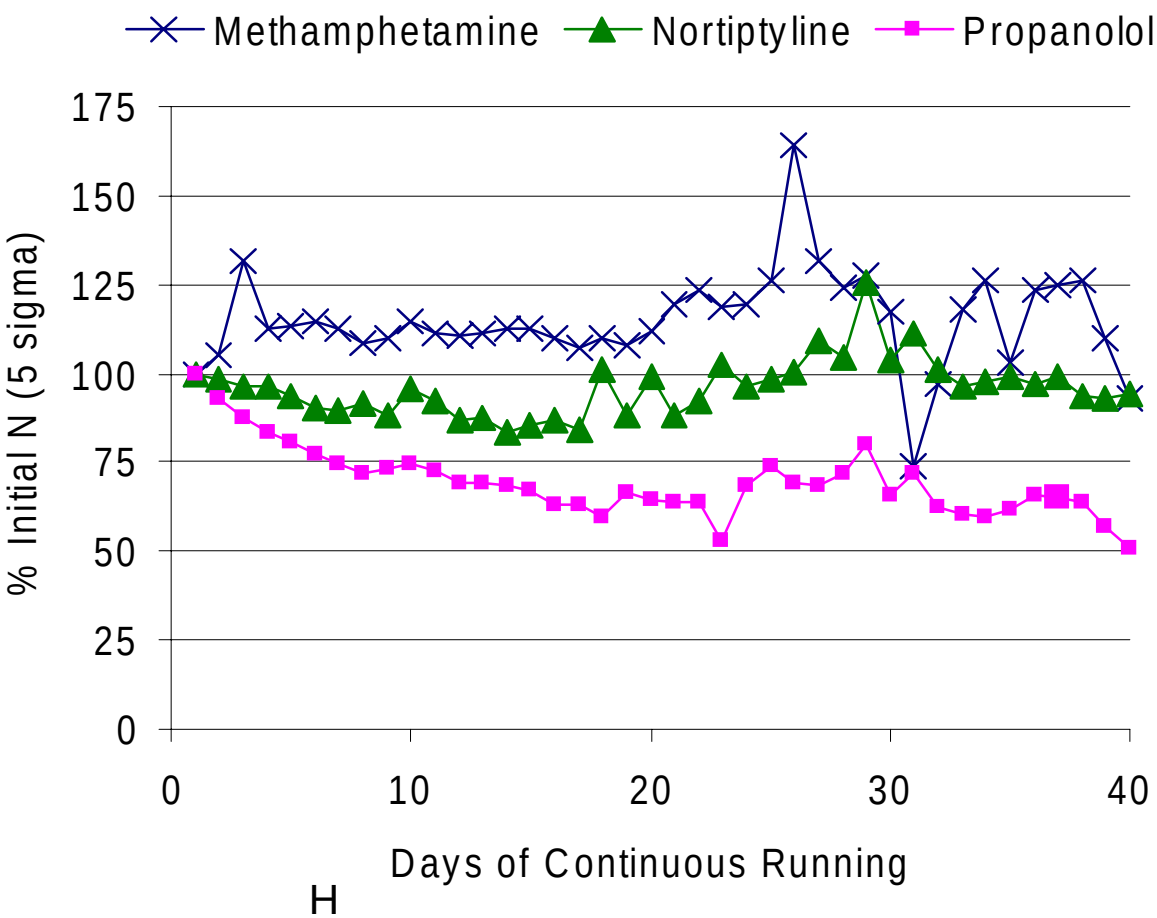
- Column : XTerra™ RP_{18}, 5µm, 4.6x150mm with an XTerra™ RP_{18}, 5µm, 3.9x20mm guard column
- Mobile phase : 50 mM Pyrrolidine- HCl pH 11.5 / MeCN, 50:50
- Column Temperature : 30 °C
- Flow Rate : 1 mL/min.
- Detector : 215 nm
- Injection volume : 2 µL

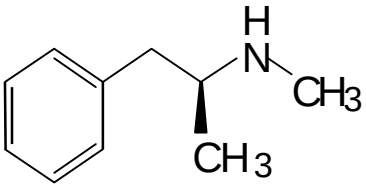
pH 11.5 Lifetime Test Chromatograms



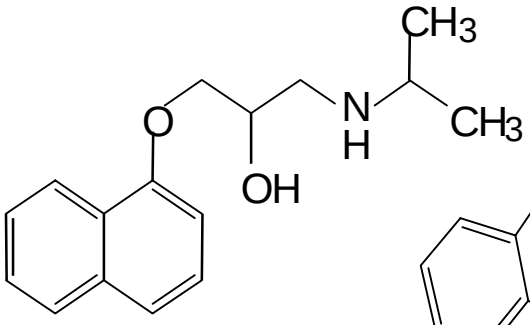
– Hybrid technology extended the high pH column lifetime of XTerra™ packing to10 times longer than C18-Silica S.

pH 11.5 Lifetime Test Efficiency *vs.* Time Curves

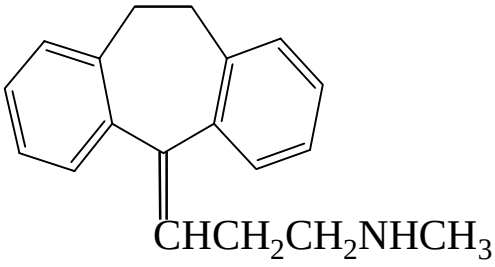




1. Methamphetamine, pK_a 9.5



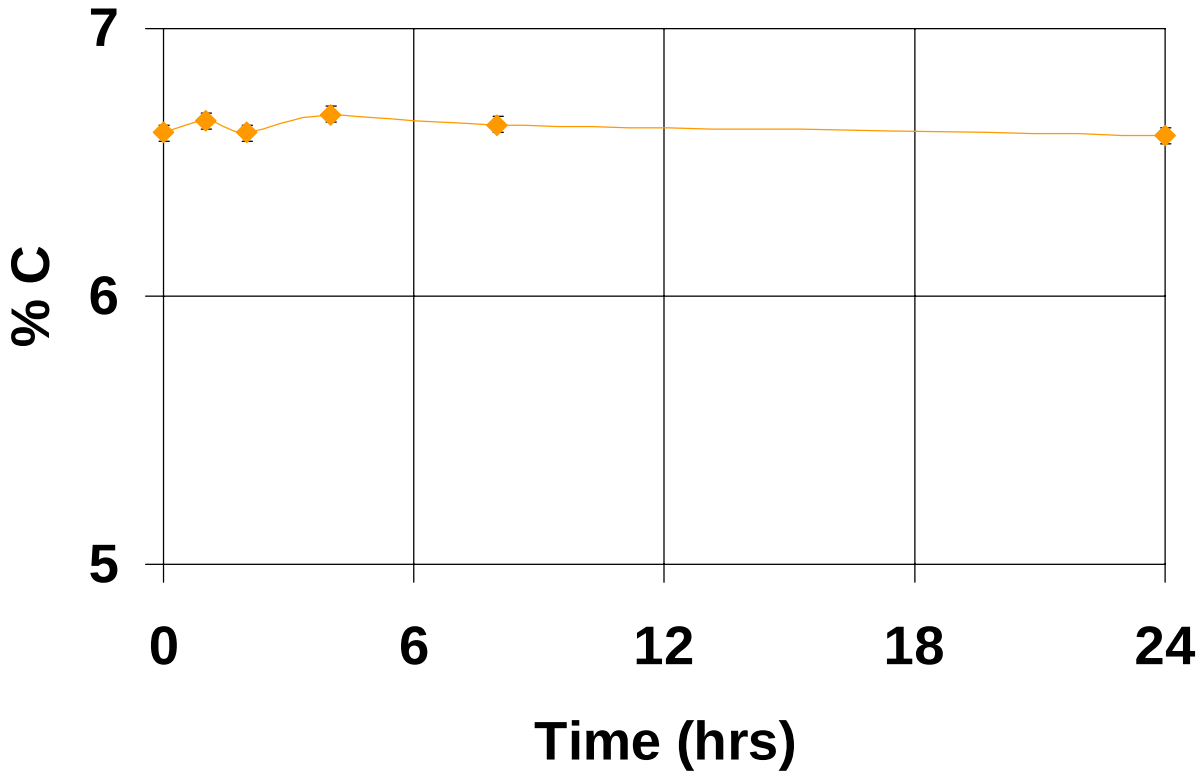
2. Propranolol, pK_a 9.5



3. Nortriptyline, pK_a 9.7

Low pH Stability of Hybrid Particle

Particle % C vs Time Exposed to 1 M HCl at 95°C



–The low pH stability of the methylsiloxane unit in the hybrid particle was tested using strongly acidic high temperature conditions. No loss of the methylsiloxane units was detected.

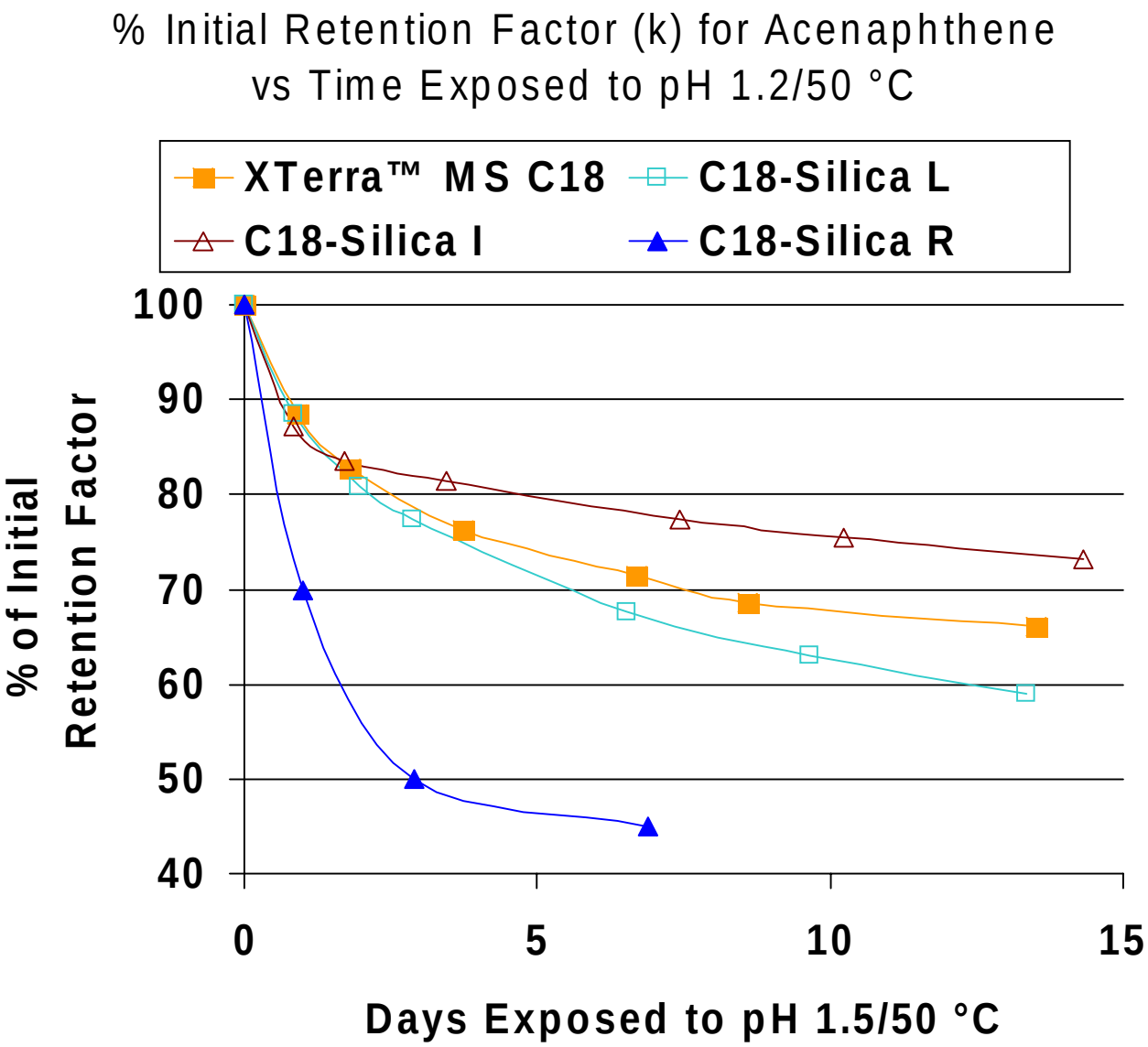
XTerra™ COLUMNS

pH 1.5 TFA/50 °C Accelerated Test Protocol

Accelerated Test Procedure:

1. **Test**: MeOH/ 20 mM K_2HPO_4 pH 7.0, 65:35 v/v, 25 °C, Flow rate 1.0 mL/min
2. **Purge:** 1% trifluoroacetic acid (TFA) pH 1.2 , Flow rate: 2 mL/min
   - 12 minutes for 4.6x75mm columns
   - 24 minutes for 4.6x150mm columns
1. **Challenge**: 1% trifluoroacetic acid pH 1.2 at **50 °C (no flow)**
2. **Purge**:
   - water at 2.0 mL/min for 10 min
   - methanol at 2.0 mL/min for 10 min
3. **Test**: MeOH/ 20 mM K_2HPO_4 pH 7.0, 65:35 v/v, 25 °C, Flow rate 1.0 mL/min
4. Cycle back to step 1 and repeat until column fails.

pH 1.5 TFA/50 °C Accelerated Test Retention *vs.* Time Curves



– Loss of retention is due to the hydrolytic loss of bonded phase.