Simplified Column Selection and Method Development

MAXPEAK™ PREMIER

Column Selection Guidance

Choosing the Right Particle

BEH Technology™	CSH™ Technology
Fully Porous	**Fully Porous**
Unparalleled pH, mobile phase and temperature versatility.	Controlled surface charge. Unparalleled peak symmetry for bases in formic acid.

HSS Technology	Solid-Core Technology
Fully Porous	**Solid-Core**
Mechanical stability of pure silica particle. Increased retention.	Highest efficiency, resolution and speed with the lowest back pressures compared to fully porous particles.

Choosing the Bonded Ligand

→ **C_{18} Phases** – Widely applicable ligand. Mid-coverage ligand (T3) ideal for polar retention. Used in some mixed mode columns as well.

→ **C_8 Phases** – Similar selectivity to C_{18} phases. Less hydrophobic than C_{18} reducing elution time. General purpose phase.

→ **Sheild RP_{18} Phases** – 100% Aqueous compatible. Different selectivity to alkyl C_{18} phases, especially for polyphenolic compounds. Improved peak shapes for basic an-alytes at/or near neutral pH.

→ **Fluoro-Phenyl (PFP) Phases** – Multiple secondary interactions between phase and analytes drive selectivity. Alternative selectivities when using Acetonitrile vs Methanol.
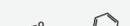
→ **Phenyl-Hexyl Phases** – Alternative selectivity when using Acetonitrile vs Methanol. Pi-Pi interactions with phenolic com-pounds drive unique selectivity.
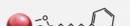
→ **Phenyl-Hexyl Phases** – Alternative selectivity when using Acetonitrile vs Methanol. Pi-Pi interactions with phenolic com-pounds drive unique selectivity.

### Three Columns to Get You Started with Method Development

BEH C_{18}
- Rugged base particle for both high and low pH analyses.
- Widely applicable ligand.
- Widely cited stationary phase for a variety of workflows.

CSH Phenyl-Hexyl
- High pH stable base particle with slight IEX characteristics.
- Alternative selectivity provided by ligand.
- Can see different retention mechanisms due to IEX functionality of particle, and secondary interac-tions of ligand.
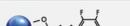
HSS PFP
- Highly retentive base particle, but not high pH compatible.
- High selectivity ligand.
- Differing retention and selectivity of this stationary phase compliments the aother two phases well.

That Final Column: A Sample Dependent Decision

#### HSS™ T3
- Highly retentive base particle, but not high pH compatible.
- Low ligand density C_{18} improves polar retention.
- 100% aqueous compatible (low strength mobile phase improves retention further).
- Well document stationary phase for polars.

BEH C_{18} AX
- Rugged and robust column for high and low pH.
- Low ligand density C_{18} improves retention of polar neutrals while mixed mode ligand retains acidic polar analytes.
- 100% aqueous compatible (low strength mobile phase improves retention further).
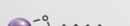
BEH C_8
- Rugged and robust column for high and low pH.
- C_8 ligand less hydrophobic, reducing retention of analytes.
- Suitable for high and low pH allowing an analyst to use appropriate conditions to elute analytes.

Method Development Approach

Choosing the Right Conditions

Define Sample and Separation Criteria

Rapid Scouting C_{18} Column | Standard ACN Gradient | Low/High pH

Low pH (~3)	High pH (~10)

Goals of Step: Assess Retention. If no retention, try HILIC or Mixed-Mode (if applicable)

Column Screening 4–6 Columns | ACN & Methanol

Acetontrile				Methanol			
Column 1	Column 2	Column 3	Column 4	Column 1	Column 2	Column 3	Column 4

Goals of Step: Determine best combination of mobile phase/stationary phase

Optimization (as needed)

Gradient Slope	Column Temperature	pH

(small adjustments)

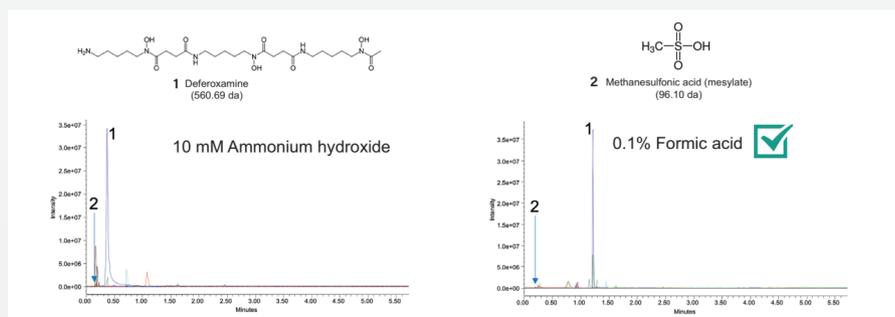**Systematic Screening Approach Benefits:**
- Tiered approach reduces decision making.
- Fast method development compared to full factorial approaches.
- Column screening after pH decision allows for rapid column selection within a narrowed down parameter.

Define Sample and Separation Criteria:
- Know when method development is finished.
- Set realistic goals for the method based on best practices in the lab.
- Select instrument and column configurations to maximize overall performance.
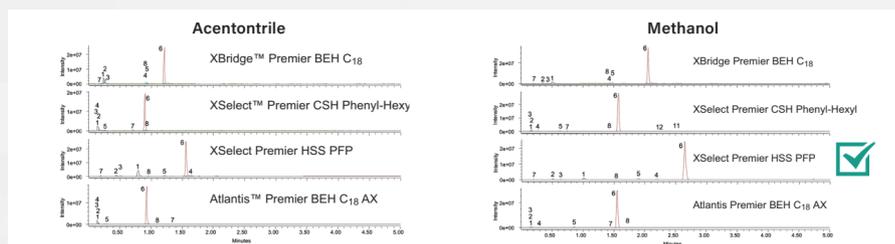
Case study sample is the forced degradation of deferoxamine mesylate using both acidic and basic stressors, heated to 70° C. Peaks tracked by mass Separation criteria defined as full separation of all analytes detected, symmetrical peak shapes, and ideally retention of mesylate.

Rapid Scouting C_{18} Column | Standard ACN Gradient | Low/High pH

1 Deferoxamine (560.69 da)
2 Methanesulfonic acid (mesylate) (96.10 da)

10 mM Ammonium hydroxide
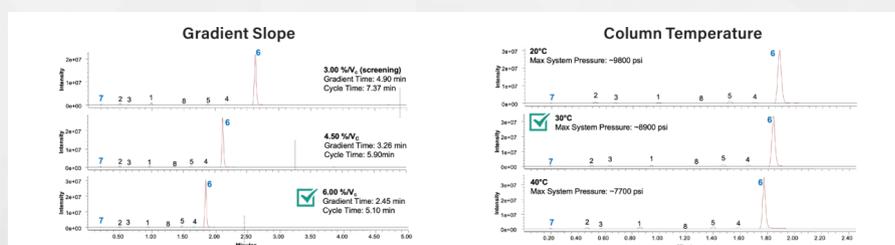
0.1% Formic acid

For this example, forced degradation of deferoxamine mesylate, pH switching shows that the two known compounds deferoxamine (1) and mesylate (2) are better re-tained at low pH. Low pH selected for the next step of the method development process, with columns being selected based on guidance in left hand panel.

Column Screening 4–6 Columsn | ACN & MeOH

Acetontrile
- XBridge™ Premier BEH C_{18}
- XSelect™ Premier CSH Phenyl-Hexy
- XSelect Premier HSS PFP
- Atlantis™ Premier BEH C_{18} AX

Methanol
- XBridge Premier BEH C_{18}
- XSelect Premier CSH Phenyl-Hexyl
- XSelect Premier HSS PFP
- Atlantis Premier BEH C_{18} AX

Column screening performed and showed that the XSelect Premier HSS PFP using methanol mobile phases provided the best separation, meeting all criteria.

Optimization (as needed)

Gradient Slope
- 3.00 %V_c (screening) Gradient Slope: 4.90 min Cycle Time: 7.37 min
- 4.50 %V_c Gradient Time: 3.26 min Cycle Time: 5.90min
- 6.00 %V_c Gradient Time: 2.45 min Cycle Time: 5.10 min

Column Temperature
- 20°C Max System Pressure: ~9800 psi
- 30°C Max System Pressure: ~8900 psi
- 40°C Max System Pressure: ~7700 psi

Optimization not required for this sample, but can still be performed as time allows. Gradient slope optimization reduced cycle time of the method by just over 2 minutes. Column temperature optimization shows slight changes in retention and system pressure, but not significant differences.

Waters™