Degradation of Samples Due to Hydrolysis on HPLC Columns - HPLC Primer

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What Is Sample Degradation by Hydrolysis in HPLC Columns? (and How TYPE-C™ Columns Mitigate It)

Hydrolysis is a common degradation pathway for many analytes during HPLC analysis; it is facilitated by acids or bases in solution and by reactive surfaces of solids that contact the liquid phase—including the stationary phase inside a column.

In several application areas (e.g., natural products, bio-actives, forensic analyses), 10–20% of bio-active compounds have been shown to hydrolyze “on-column” under typical chromatographic conditions, which can compromise accuracy, quantitation, and impurity profiling.

Why Hydrolysis Happens Inside Conventional Silica Columns

- High surface area intensifies contact: Both irregular and spherical silica phases are engineered with very high surface-to-volume ratios. Greater surface area → more surface interactions → higher likelihood of on-column chemical change.
- Silanol (Si-OH) activity persists even when “fully end-capped”: Conventional phases can still present ~30–50% free silanols, which can act as acidic/interactive sites that facilitate analyte hydrolysis or secondary retention.
- You see it in your data: The prevalence of on-column hydrolysis has been highlighted by PDA (photodiode array) and LC-MS measurements, where degradation products appear and increase over repeated injections or at certain pH conditions.

How Cogent TYPE-C™ (Silica Hydride) Columns Change the Equation

Cogent TYPE-C™ materials replace the conventional hydroxylated silica surface with a silica-hydride (Si–H) surface. As a result, the packing is much less hydrolytically active than ordinary silica bearing Si-OH groups.

In many cases, the parent compound does not hydrolyze on the column, enabling you to track injected quantities rather than degradation products—a major advantage for non-targeted or stability-sensitive analyses.

Key takeaway: *By minimizing acidic/interactive surface sites, TYPE-C™ columns can reduce on-column hydrolysis and improve fidelity between what you inject and what you detect—especially valuable when screening unknowns or labile bio-actives.*

What This Means for Method Developers

- If you observe unexpected degradants or mass balances that don’t add up, consider on-column hydrolysis as a root cause—especially with acid/base mobile phases and silica phases with appreciable silanol activity.
- For sensitive compounds (esters, lactones, some pro-drugs, acylated natural products, etc.), migrating from conventional silica to Cogent TYPE-C™ can help preserve the native molecule through the separation.

At-a-Glance: Conventional Silica vs. TYPE-C™ for Hydrolysis-Prone Analytes

- Conventional silica (Si-OH rich) → high surface area + residual silanols (even after end-capping) → greater risk of on-column hydrolysis; degradants frequently confirmed by PDA/LC-MS.
- Cogent TYPE-C™ (Si–H surface) → much lower hydrolytic activity; in many cases no on-column hydrolysis observed, enabling more faithful quantitation of the parent.

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