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Trends in HPLC – Wide pH-Range Columns and Capillary Columns

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Abstract: The topic covers the current trends in HPLC column development to robust columns that extend the usable pH-range. Examples are given on how intelligent surface chemistry enables silica-based materials to be used in the pH-range 1 to 11.

The resulting packing materials combine the extreme mechanical stability of silica with an excellent chemical stability. Especially in the LC-MS community high pH-applications are becoming more popular, because of the problems that ion-pair reagents cause with this detection technique.

The life-science arena with small sample amounts demands smaller id columns *i.e.* capillary columns. Capillary-HPLC instruments are now commercially available and the presentation will shed some light on various capillary column applications.

Keywords: Capillary-HPLC · High pH · Inert RP material · LC-MS · Peptide

During HPLC method development and the selection of the stationary phase, stability at different pH levels is often ignored. If this happens a sub-optimal stationary phase is selected and the robustness of the method affected. The stability of reversed phase packing material is attacked in two ways:

- at low pH the organic layer like C18 cracks off by hydrolysis,
- at high pH insufficient coverage of the organic layer makes silica dissolution possible.

Bonded phase chemistry that gives best results at low pH might be not the right choice for high pH. The need to run samples at high pH resulted in the development of polymeric particles. They show excellent pH-stability but a lack of mechanical stability.

A series of RP materials based on silica was developed for optimum performance at various pH-ranges [1]:

- For acid conditions Zorbax SB is the best choice (Fig. 1).

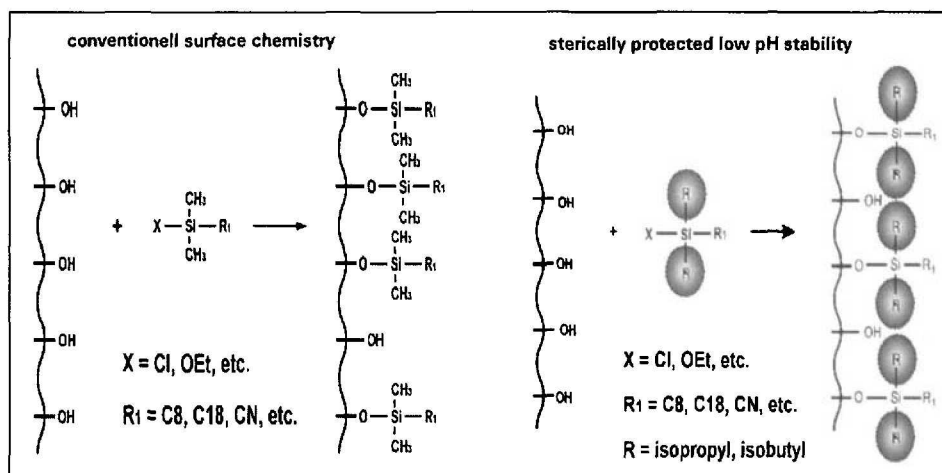


Fig. 1.

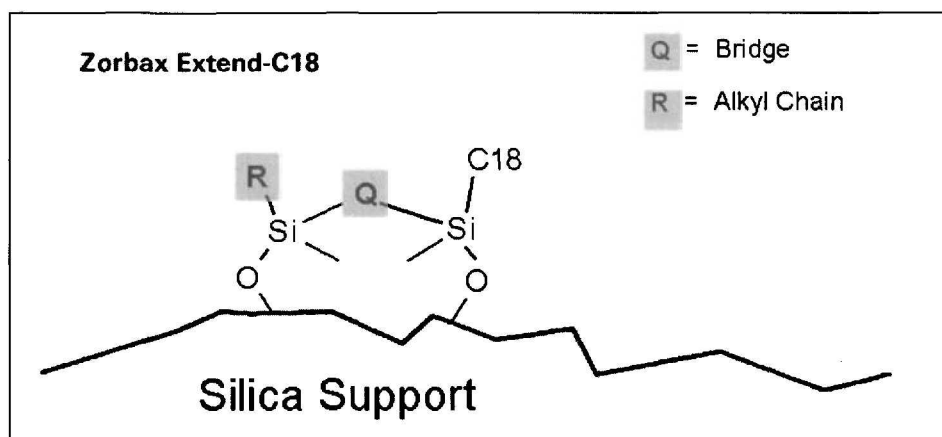


Fig. 2.

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- For maximum column lifetime up to pH 12 the Zorbax Extend was developed (Fig. 2). The excellent stability is demonstrated in Fig. 3.
- A practical application, the separation of Angiotensines, shows the benefit of running a chromatogram at high pH (Fig. 4).
- For polar compounds that need to be analyzed at a high water content in the mobile phase, Zorbax Bonus-RP and the Zorbax-Aq are available (Fig. 5).

The robustness of the capillary HPLC method with modern equipment is demonstrated in Fig. 6. This is no longer a technique only for scientific research, but for routine applications with limited sample amounts or when LC/MS detection is needed.

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[1] B.E. Boyes, 'Separation and Analysis of Peptides at High pH Using RP-HPLC/ESI-MS', 4th Symposium on the Analysis of Well Characterized Biotechnology Pharmaceuticals, San Francisco, CA January 9–12, 2000.

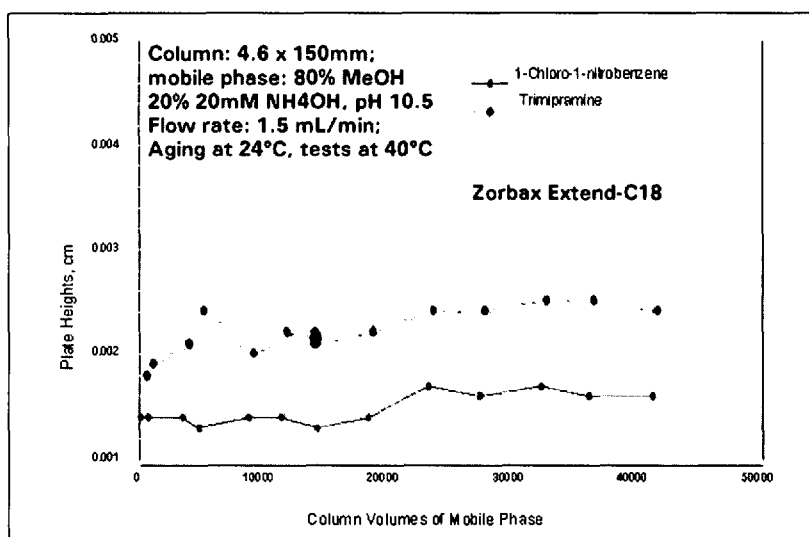


Fig. 3.

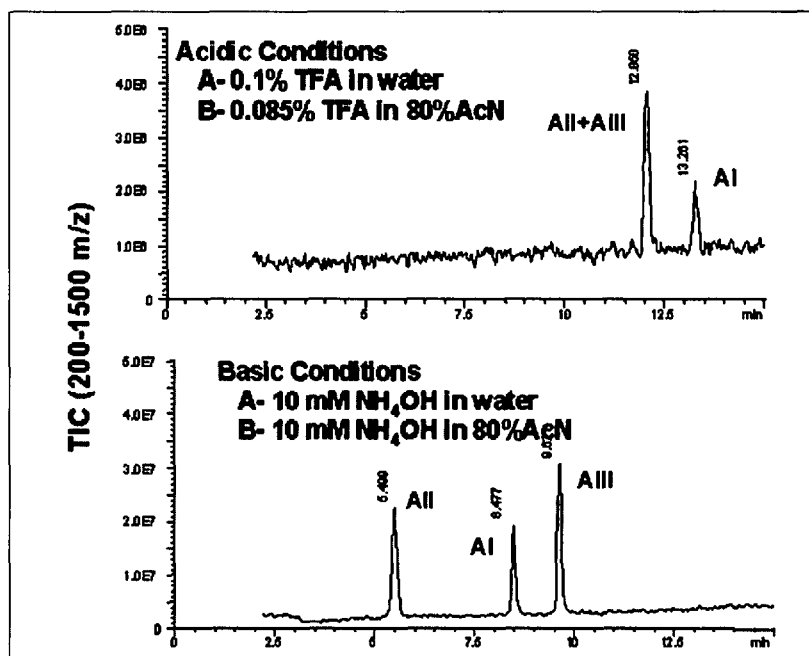


Fig. 4.

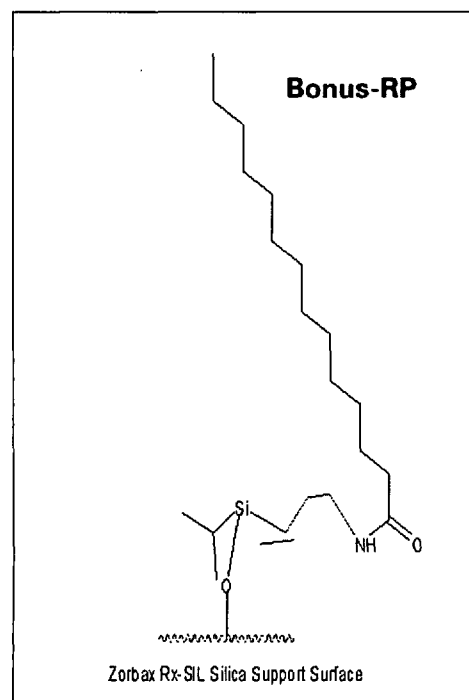


Fig. 5.

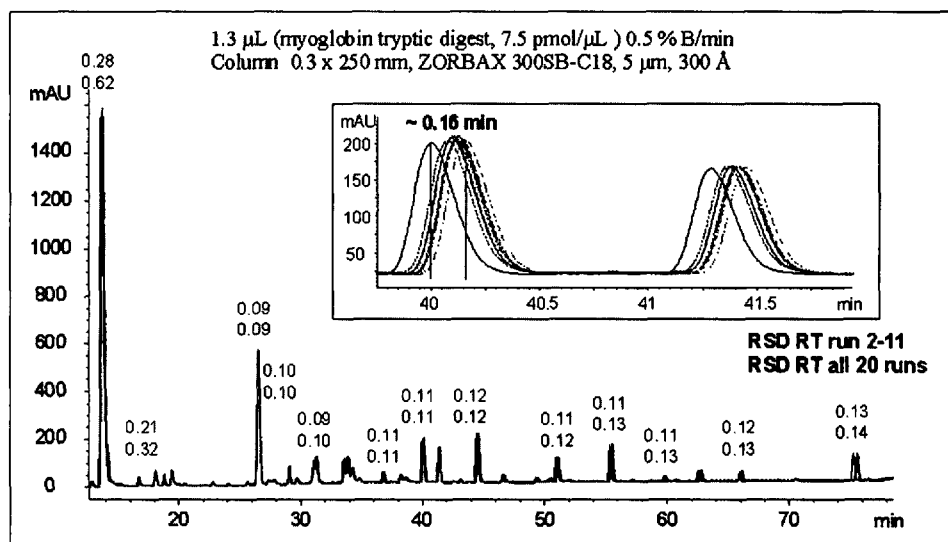


Fig. 6.