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Introduction

Reversed phase HPLC is a commonly used purification technique within downstream processing of therapeutic peptides and smaller proteins. Repetitive injections of large amounts of crude peptide often require periodic washing or regeneration procedures (CIP) of the column packing material. While in most cases a washing step with a high content (>70%) of organic modifier is able to desorb and elute highly hydrophobic species from the column, it is also possible that an acidic or alkaline treatment is required in order to regenerate the packing material. NaOH denatures the eventually aggregated peptide or protein species, which is a prerequisite for eluting them with a high content of organic modifier. Caustic treatments (NaOH) are very common and uncontested for polymeric packing materials. In the case of silica based materials, NaOH treatments that render pH > 10 bring along an intrinsic risk of hydrolyzing siloxane bonds in the silica matrix, which are the backbone of the porous structure¹. Continuous hydrolysis leads not only to deteriorating column performance, but also species used for surface modification (silanes) will elute from the column and are likely to contaminate product fractions in the case of preparative separations. Such leachables from the stationary phase are devastating for preparative chromatography and are gaining more and more attention from e.g. FDA when approving drug manufacturing processes.²

It is well known, that features such as high ligand density and in some respect end-capping offer a reasonable protection against alkaline hydrolysis³. Within this study, we compared several commercially available C18 modified silica-based packing materials upon their chemical stability under NaOH regeneration conditions. Furthermore, we compared C4, C8 and C18 modified materials with each other.

Test Idea

The columns (4,6x250 mm) were subjected to alkaline elution conditions and potential hydrolysis of the surface modification and / or the silica backbone was monitored by an appropriate chromatographic test. Furthermore, the eluents were analyzed upon their silicon content by ICP-AES.

Experimental conditions

Elution Conditions:

Eluent: 1. Ethanol (99.5 %) / 1 mM NaOH (aq) 50:50 (v/v)
2. Ethanol (99.5 %) / 10 mM NaOH (aq) 50:50 (v/v)
3. Ethanol (99.5 %) / 100 mM NaOH (aq) 50:50 (v/v)
Flow rate: 1.0 mL/min
Amount: 3 x 10 column volumes

Chromatographic test

Mobile phase: Methanol: 25 mM KH₂PO₄ pH 7.0: 80:20 (v/v)
Flow rate: 1.0 mL/min
Sample solution: nortriptyline, toluene, imipramine, amitriptyline, uracil,
Detection: 215 nm

Procedure:

- Chromatographic test
- The column was equilibrated with 10 column volumes of 100 % ethanol.
- The column was purged with 11 column volumes of eluent 1 and column volumes 2-11 were collected in a PP bottle. The same was repeated for eluents 2 and 3
- The column was purged with 10 column volumes ethanol / H₂O / HAC 10:90:0.2 (v/v/v).
- The column was purged with 11 column volumes 100 % acetonitrile. Column volumes 2-11 were collected in a PP bottle.
- Chromatographic test
- The collected eluents were analyzed upon their silicon content by ICP-AES

Results

As can be seen in figure 1, the NaOH treatment rendered accessible silanol groups that lead to an increase of the retention time for amitriptyline. This is the case for all packing materials tested, no significant difference can be seen. However, when analyzing the eluents upon their silicon content, the different C18 modified materials differ drastically in their susceptibility for silicon leakage (figure 2). None of the C18 modified materials shows any Si-leakage at 1 mM NaOH (pH 11). At pH 12 (10 mM NaOH) most C18 modified materials showed leakage in the range of 10-30 ppm Si. At 100 mM NaOH (pH 13), which is the concentration

generally necessary for CIP treatments, all the materials show Si leakage however at very different levels, between 30 and 520 ppm. Furthermore, the comparison between C4, C8 and C18 modified materials shows clearly, that C18 modified silica offers the best protection towards alkaline hydrolysis.

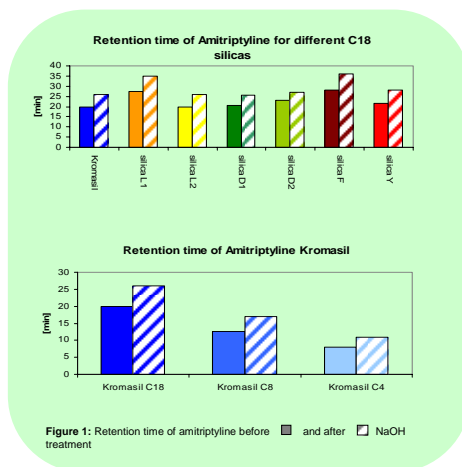


Figure 1: Retention time of amitriptyline before and after NaOH treatment

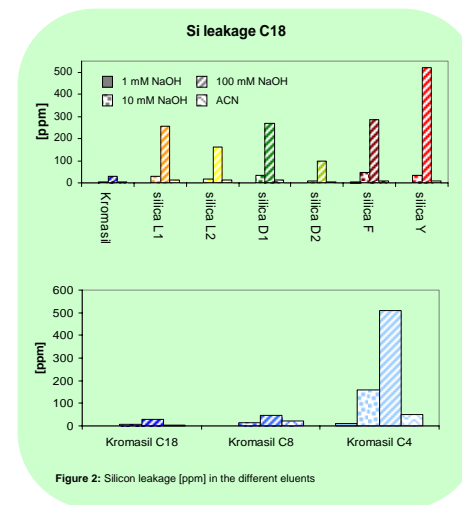


Figure 2: Silicon leakage [ppm] in the different eluents

Discussion and Conclusion

While chromatographic tests hardly reveal any differences in the chemical stability of different RP silicas under alkaline conditions, the analysis of the eluents shows clearly that there is a correlation between the carbon content (table 1) and the silicon leakage (figure 2). C18 materials that have a high carbon content and thus high ligand density offer best protection towards alkaline hydrolysis. KR C18 with 20.1% C showed a leakage of 33 ppm Si under 100 mM NaOH while for silica Y with 16.6% C, 520 ppm Si were determined. These findings are in good agreement with literature³.

Based on these results it is recommended to use high ligand density C18 silicas if NaOH treatment is needed for the regeneration of the column.

Table 1: Specific surface area and carbon content for different packing materials.

	KR C4	KR C8	KR C18	L1	L2	D1	D2	F	Y
SSA [m ² /g]	327	326	329	410	394	299	456	270	345
Carbon content [%]	7.9	12.1	20.1	18.3	17.6	17.1	17.3	16.4	16.6

References

- 1 H. A. Claesson et al.; J. Chromatogr. A; 728 (1996) 259-270
- 2 L. L. Ng, Oral presentation L-105 at PREP 2005, Philadelphia, PA, USA
- 3 J. J. Kirkland et al.; J. Chromatogr. A; 797 (1998) 111-120