Electronic Supplementary Material (ESI) for Analytical Methods.

Supplement

Quantification of N-hydroxysuccinimide and N-hydroxysulfosuccinimide by hydrophilic interaction chromatography (HILIC)

Oleg Klykov and Michael G. Weller*

BAM Federal Institute for Materials Research and Testing, Division 1.5 Protein Analysis,
Richard-Willstaetter-Strasse 11, 12489 Berlin, Germany

* Corresponding author. Tel. +49-30-8104-1150; fax: +49-30-8104-1157.
E-Mail address: michael.weller@bam.de (M. G. Weller)

Introduction

Inspired by the findings of Fountain et al. [25], we decided to explore more basic conditions for the separation. In this paper, it was reported that weak acids highly profit from a higher pH, showing better retention and better response in mass-spectrometry.

Due to a pK_a value around 6.0, it was assumed that a higher pH of the solvent might be advantageous for full deprotonation of NHS. Since a basic pH might damage most silica-based HPLC columns, a polymer-based, zwitterionic column was tested.

Chromatographic method based on a polymer-based HILIC column

A zwitterionic, polymer-based HILIC column (Merck, SeQuant zic-pHILIC, 150 mm x 2.1 mm, 5 µm), was used for the isocratic determination of NHS samples in a 100 mM ammonium hydroxide buffer (pH 11). The mobile phase consisted of 90% acetonitrile and 10% of 10 mM aqueous ammonium hydroxide (pH 11, before mixing). The column temperature was set to 30°C. The system was equilibrated for 10 minutes before each run. The flow rate was 0.4 mL/min and the injection volume was 1 µL. Detection was performed by UV at 220 and 260 nm; the total run time was 20 min.

Determination of NHS on a polymer-based HILIC column

An important aspect, which was also discussed in [25] is the influence of the solvent concentration on the pK_a of a compound. It is expected that weak acids become even weaker, which was exactly what we observed in our experiments. Ammonium acetate with high concentrations of acetonitrile was not able to deprotonate NHS completely. This means that more basic buffers are needed to lead to a quantitative deprotonation of NHS. Protonated NHS shows a weaker retention on HILIC columns, the absorbance maximum shifts from the characteristic peak at 260 nm of the anion to shorter wavelengths (220 nm), which might lead to more interferences. Unfortunately, most silica columns do not withstand high pH conditions. Therefore, special columns have to be used for this purpose. Materials based on polymeric beads are particularly useful for this kind of applications.

Unfortunately, they often show less favorable separation efficiencies than their silica counterparts. Another option might be the use of hybrid silica columns, which are also stable at quite high pH (not tested).

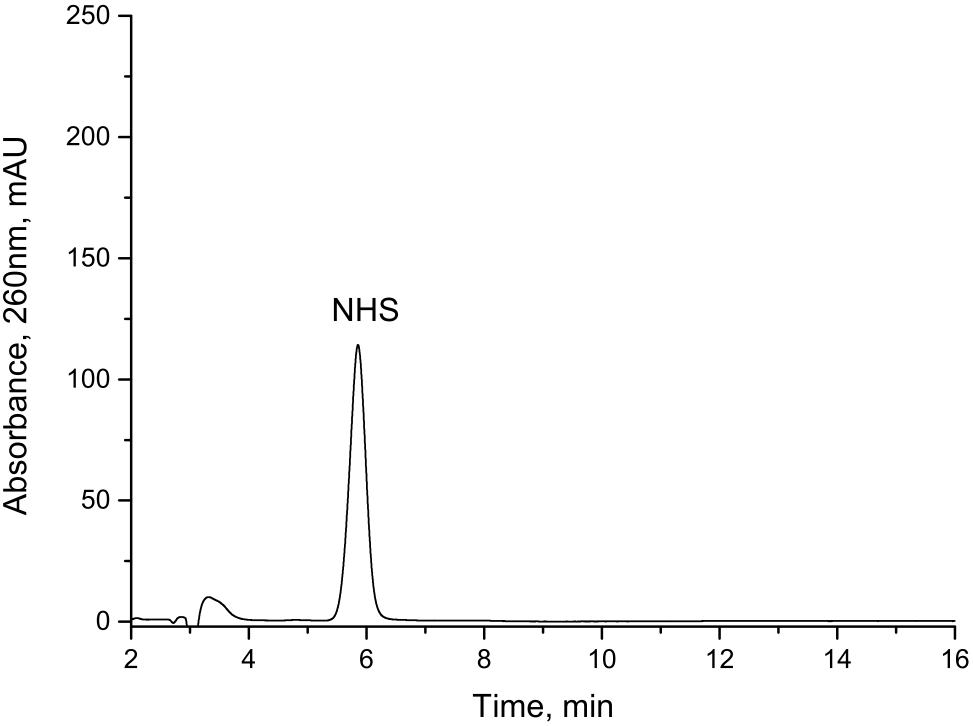

Fig. 1S: Separation of NHS on a polymer-based HILIC column (isocratic, 10% 10 mM ammonium hydroxide, pH 11, 90% acetonitrile, 30°C), sample was 1 µL of 200 mg/L NHS.

Conclusions

In this supplement, it could be shown that the application of polymeric HILIC columns with mobile phases at high pH is possible. However, since the peak on the polymeric phase seems to be broader, which might be mainly caused by the larger particle diameter, the slight increase of the apparent extinction coefficient may be not worth the effort. A UV detection at 220 nm and 260 nm is possible, the latter being potentially less prone to spectral interference. Unfortunately, no significant advantage of the polymer-based column was found in these experiments. Therefore, the validation of the HILIC method was limited to the silica-based system (see main paper). Nevertheless, it could be shown that the application of a polymer-based column at high pH is possible, which might be more beneficial in the case of MS detection as reported in [25]. However, it should be considered that the high-pH approach most likely is not suitable for NHS ester analysis, which would be quickly cleaved under these conditions.

Acknowledgements

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Reference

[25] K.J. Fountain, J. Xu, D.M. Diehl, D. Morrison. Influence of stationary phase chemistry and mobile-phase composition on retention, selectivity, and MS response in hydrophilic interaction chromatography. J. Sep. Sci. 33 (2010) 740-751.