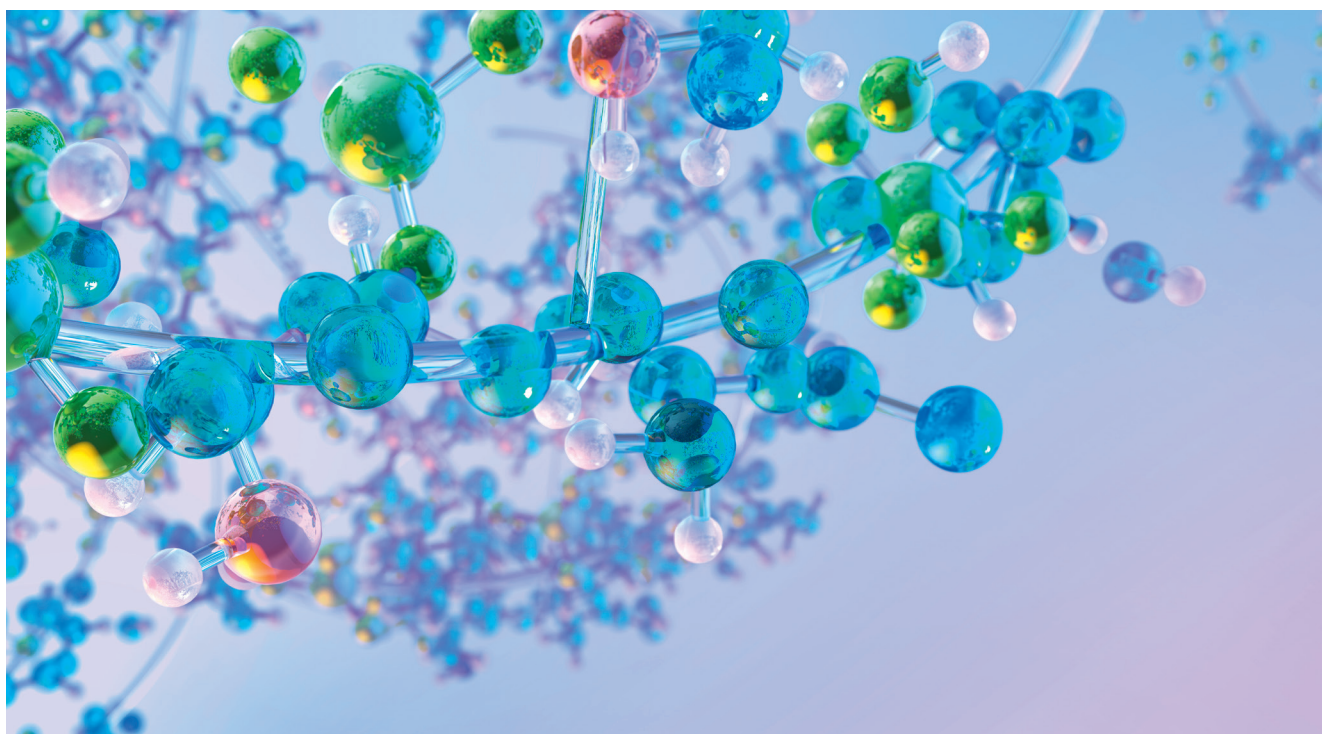


How to protect your peptides from oxidation during HPLC analysis

When analysing biomolecules with standard HPLC systems and columns, undesirable side effects can occur due to interactions with metal surfaces. Many system components and conventional column hardware are made of stainless steel. Eluents such as methanol or acetonitrile in high concentrations can lead to surface corrosion – even on titanium components [1].

The resulting positively charged surface can interact with the analytes [2], which is particularly problematic for peptides and proteins. Unpaired cysteine and methionine residues are especially susceptible to oxidation [3]. This then leads to adulterated results because the oxidised peptide fractions are more polar and therefore elute earlier on reversed-phase columns.



Possible sources of oxidation

Catalytic metal residues can enter the system from various sources. Digestion buffers, components of the HPLC system, and the analytical column can all induce peptide oxidation. For example, phosphate buffers typically contain higher levels of residual metal ions than Tris buffers. Injecting multiple times from a single vial can cause iron to enter the sample due to metal leaching from the injection needle. Furthermore, long-term use of a column with typical eluents like acetonitrile and methanol leads to metal leaching and an accumulation of metal residues on the stationary phase in the column.

The example from Zang et al. [4] (Fig. 1) shows the oxidation peptide map induced by two different columns with different column history. The monitored peptide contains two methionine residues, where oxidation results in two separate peptide oxidation peaks (Peak1 and 2). Peak3 is the main peak and Peak4 represents a deamidated species. Column#1, which has been in use longer, clearly shows more oxidation of the peptide compared to the new column#2, demonstrating that the level of residual metals increases over time. It is possible that a visual change in chromatographic behavior may not be observed until hundreds to thousands of injections have been performed [5].

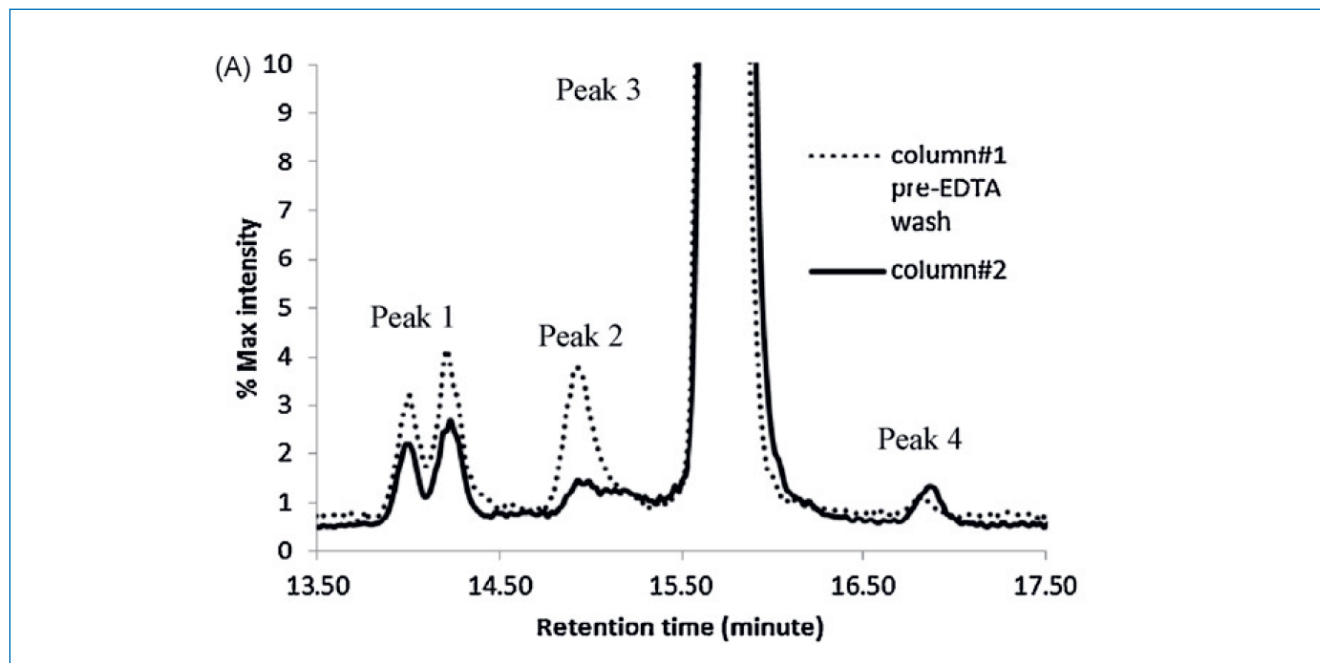


Fig. 1: Total ion chromatograms of an oxidation peptide map comparing a used column #1 with a new column #2 [4].

How to reduce peptide oxidation

Residual metals can be masked by flushing the column with an EDTA solution [6]. The example (Fig.2) shows how the chromatographic behaviour of column #1 matches that of column #2 again after an overnight column-wash with 20 mM EDTA and 10% acetonitrile solution at 0.3 mL/min (18 hours).

This procedure must be repeated regularly to ensure the quality of results. Furthermore, it is possible to add EDTA or methionine directly to the sample [7,8] to reduce oxidation effects. More passivation protocols can be found in the previous [YMC Expert Tip: Reducing adsorption on metal surfaces](#).

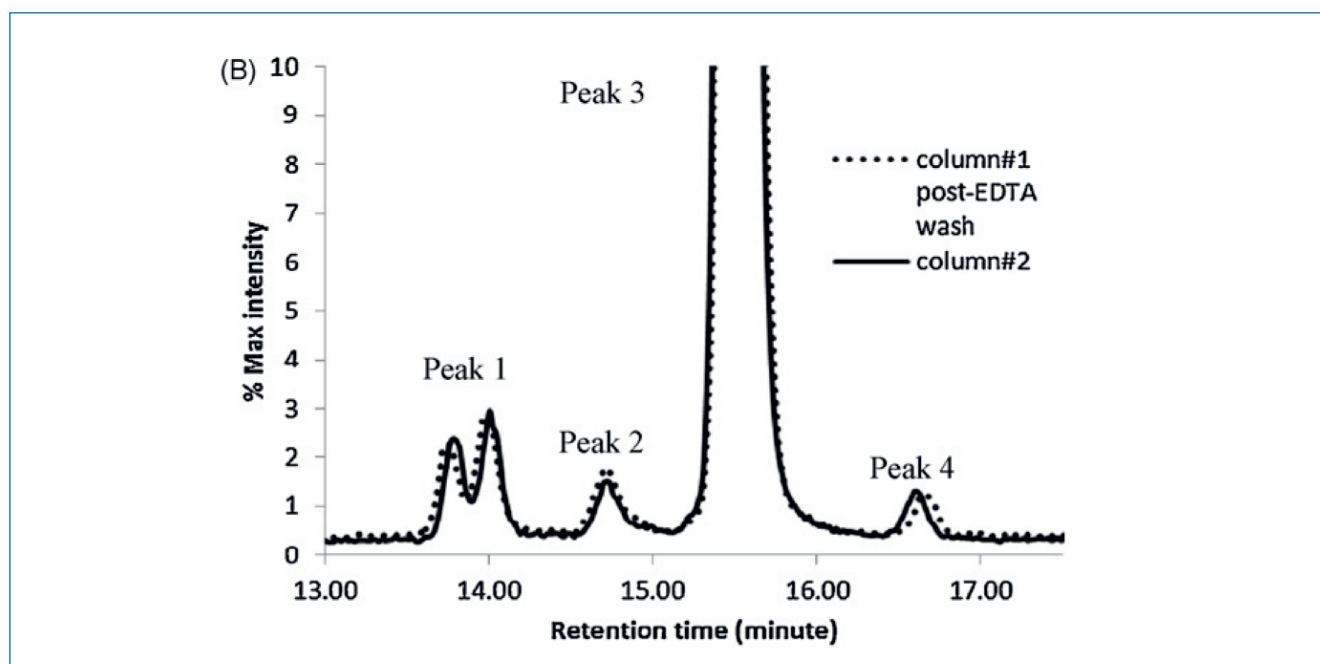


Fig. 2: Total ion chromatograms of an oxidation peptide map after an overnight EDTA wash of column #1 compared with column #2 [4].

Avoiding peptide oxidation

On-column peptide oxidation can be avoided by using a bioinert and corrosion-resistant column, such as the bioinert coated YMC Accura columns. Since the HPLC column - including the frits - provides over 70% of the surface that comes into contact with the sample, using a bioinert column will clearly reduce peptide oxidation.

However, it is recommended to use a true bioinert HPLC system that includes bioinert wetted parts. It should be noted that biocompatible systems are not suitable for avoiding peptide oxidation, as they include titanium and/or alloys that can still cause metal leaching [1].

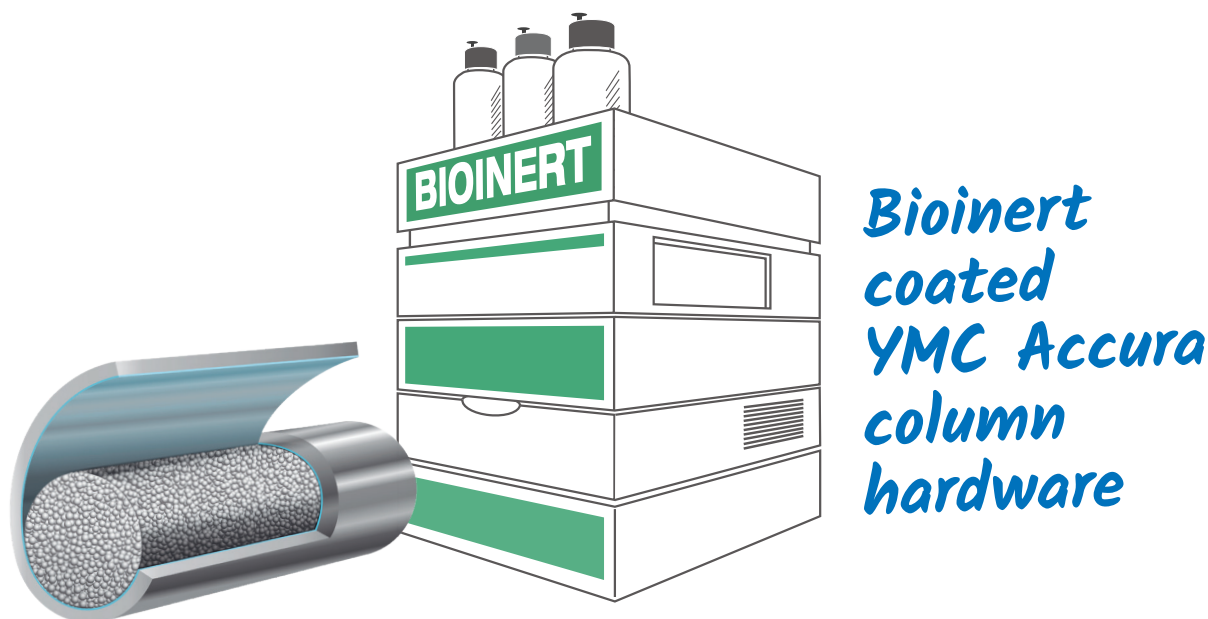


Fig. 3: Cross section of a bioinert coated YMC Accura column.

References

- [1] Mowery, R. A. *The Corrosion of 316 Stainless Steel in Process Liquid Chromatography with Acetonitrile or Methanol Carriers*. *J. Chromatogr. Sci.* 1985, 23, 22–29 DOI: 10.1093/chromsci/23.1.22
- [2] Stoll, D. R.; Hsiao, J. J.; Staples, G. O. *Troubleshooting LC Separations of Biomolecules, Part 1: Background, and the Meaning of Inertness*, *LCGC Europe* 2020, 33 (3), 122–126.
- [3] G. Xu, M.R. *Chance Radiolytic modification and reactivity of amino acid residues serving as structural probes for protein footprinting*, *Anal. Chem.* 77(14), 4549–4555 (2005) DOI: 10.1021/ac050299+
- [4] L. Zang et al., *Residual metals cause variability in methionine oxidation measurements in protein pharmaceuticals using LC-UV/MS peptide mapping*, *J. Chrom. B*, 2012,
- [5] B. Mautz, M. König, *Monitoring of On-column Methionine Oxidation as Part of a System Suitability Test During UHPLC–MS/MS Peptide Mapping*, *LCGC Supplements* 2019, 37 (11), 8–13
- [6] A. Fleitz, E. Nieves, C. Madrid-Aliste, S. J. Fentress, L. D. Sibley, L. M. Weiss, R. Hogue Angeletti, F.-Y. Che, *Enhanced Detection of Multiply Phosphorylated Peptides and Identification of Their Sites of Modification*, *Anal. Chem.* 85 (2013), 8566–8576.
- [7] M. Gilar, M. Delano, F. Gritti, *Mitigation of analyte loss on metal surfaces in liquid chromatography*, *J. Chromatogr. A* 1650 (2021) 462247.
- [8] B.C. Tiansheng Li, Christopher Sloey, *US Patent 5849700 US 2003/0104996 A1*, 2003.