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应用纪要

Low Adduct Peptide LC-MS Obtained with IonHance DFA and Certified LDPE Containers

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This is an Application Brief and does not contain a detailed Experimental section.

Abstract

This application brief demonstrates to minimize metal ion adducts in the mass spectra of peptides obtained with IonHance DFA modified mobile phases.

Benefits

Minimizing metal ion contamination for low adduct LC-MS separations using high purity mobile phase additives and containers.

Introduction

Sodium and potassium adducts have long posed a challenge to obtaining high quality protein and peptide electrospray ionization (ESI) mass spectra. While trace level metal contaminants generally do not affect protein or peptide separations, they can disrupt the interpretability of the mass spectra by distorting relative abundances of protonated species and causing spectral crowding. Sodium and potassium adducts are notoriously difficult to minimize as they can originate from multiple sources of contamination as part of sample preparation and instrument operation.

Minimizing trace metal contamination in MS based workflows has become ever more critical in protein and peptide analyses as increasingly sensitive assays are being deployed throughout the development and manufacturing of biotherapeutics. Herein, we highlight the value in combining high quality laboratory purified water and certified low-density polyethylene (LDPE) mobile-phase containers to achieve exceptionally high quality peptide mass spectra using IonHance Difluoroacetic Acid (DFA) (p/n: 186009201).

Results and Discussion

Thermoplastics such as perfluoroalkoxy (PFA) and polyethylene (PE) are favored container materials for trace metal quantitation applications like inductively coupled plasma mass spectrometry (ICP-MS). Their appeal comes from their chemical purity and inertness to acids and bases. Moreover, they are frequently certified to contain low part per billion (or even part per trillion) levels of metals. For this reason, IonHance DFA is packaged in a PFA vial and it is recommended that DFA modified mobile phases be prepared in Waters Certified LDPE containers, each of which are rigorously QC tested to ensure less than 100 ppb levels of sodium and potassium.

To assess the suitability of new Waters Certified LDPE Containers (p/n: 186009110) for preparations of IonHance DFA mobile phases, we have explored the quality of mass spectra resulting from peptide mapping separations of the Waters mAb Tryptic Digestion Standard (p/n: 186009126), a newly commercialized standard based on a tryptic digest of NIST Reference Material 8671. Figure 1 shows example UV and TIC chromatograms of the peptides from this standard as obtained with 0.1% IonHance DFA, 18.2 MΩ laboratory purified water and Certified LDPE Containers. Meanwhile, Figure 2 shows a corresponding mass spectrum for the T13 peptide from the light chain of NIST mAb. Notice with the combination of 18.2 MΩ laboratory purified water and Certified LDPE Containers (Figure 2A), the peptide spectrum can be easily interpreted. In comparison, when the mobile phase was prepared without

Certified LDPE containers and with externally sourced LC-MS grade water (Figure 2B), the sodium and potassium adduct signals are noticeably higher. Represented here, the formation of metal adducts can skew the abundance of protonated ions, which can be impactful to certain data analyses.

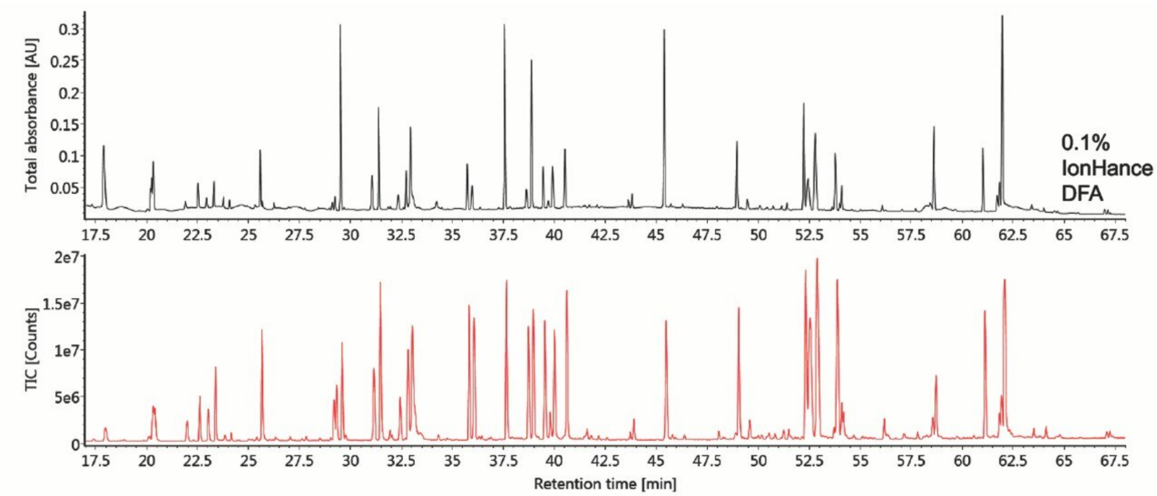

Figure 1. UV and total ion current (TIC) chromatograms from a peptide mapping separation of the mAb Tryptic Digestion Standard using 0.1% IonHance DFA modified mobile phases and an ACQUITY UPLC Peptide CSH C_{18} , 130 Å, 1.7 µm, 2.1 x 150 mm Column.

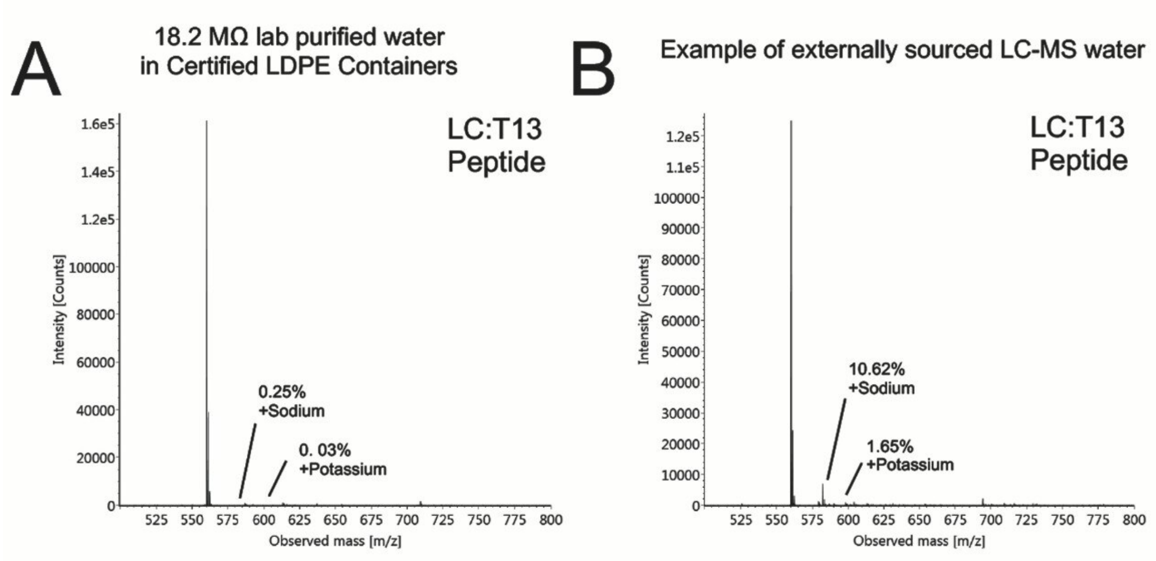

Figure 2. Extracted mass spectra of the T13 peptide (sequence VQWK) from the light chain of the mAb Tryptic Digestion Standard as acquired with 0.1% IonHance DFA modified mobile phases prepared with 18.2 MΩ lab-purified water and Certified LDPE Containers (A). An example mass spectrum from a mobile phase prepared with externally sourced LC-MS water and without Certified LDPE Containers is also shown for comparison (B).

Conclusion

While it is important to many different assays, the minimization of metal ion contamination in mobile phases can be a particularly challenging task during LC-MS experiments. It is critical to adopt stringently purified reagents and clean materials for mobile phase preparation. Successful deployment of LC-MS-based workflows require methods that employ high purity reagents and materials. Waters Certified LDPE Containers afford a reliable means to controlling sodium and potassium contamination such that high quality spectra can be obtained without interference from trace metal sodiated and potassiated ions, especially when combined with a high purity acid such as IonHance DFA.

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