

Online Hydrophobic Interaction Chromatography-Mass Spectrometry for Top-Down Proteomics

Datenbank

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Deskriptoren

Protein; Ammonium; Acetat; Interaktionschromatographie; Proteintrennung; qualitative Analyse; Ladungsverteilung; Umkehrphasenchromatographie; Proteomik; Proteinstruktur; Massenspektrum; Massenspektrometrie; mobile Phase

PROTEINS; AMMONIUM-ION; ACETATES;
INTERACTION-CHROMATOGRAPHY; PROTEIN-SEPARATION;
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MOBILE-PHASES

Abstract

Recent progress in top-down proteomics has led to a demand for mass spectrometry (MS)-compatible chromatography techniques to separate intact proteins using volatile mobile phases. Conventional hydrophobic interaction chromatography (HIC) provides high-resolution separation of proteins under nondenaturing conditions but requires high concentrations of nonvolatile salts. Herein, we introduce a series of more-hydrophobic HIC materials that can retain proteins using MS-compatible concentrations of ammonium acetate. The new HIC materials appear to function as a hybrid form of conventional HIC and reverse phase chromatography. The function of the salt seems to be preserving protein structure rather than promoting retention. Online HIC-MS is feasible for both qualitative and quantitative analysis. This is demonstrated with standard proteins and a complex cell lysate. The mass spectra of proteins from the online HIC-MS exhibit low charge-state distributions, consistent with those commonly observed in native MS. Furthermore, HIC-MS can chromatographically separate proteoforms differing by minor modifications. Hence, this new HIC-MS combination is promising for top-down proteomics.

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