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Size-exclusion chromatography using reverse-phase columns for protein separation

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Highlights

- Mass spectrometry-compatible, protein SEC separation using reverse-phase columns.
- Reverse-phase columns can separate most proteins based on molecular weight.
- A solution for non-specific binding occurred in conventional SEC--RP-based SEC.

Abstract

Reverse-phase (RP) liquid chromatography (RPLC) and size-exclusion chromatography (SEC) are methods commonly used for protein/peptide separation, and they are based on distinct principles. This study develops a method using RP columns for size-based separation of protein mixtures. Results show that high concentrations of acetonitrile with trifluoroacetic acid as an acid modifier successfully suppressed interactions between proteins and the stationary phase and allowed the RP column to act as a SEC column to separate proteins based on their molecular weight. The reduction of protein disulfide bonds resulted in an improved

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