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Novel combinatorial ligand for antibody separation: Peptide ligand with hydrophobic charge-induction group

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Affinity biomimetic chromatography with peptide ligands is a novel technology for antibody purification, which has the advantages of high specificity, stable ligand structure and low cost. However, due to high affinity of specially-designed ligands for antibody molecule, harsh conditions often have to be used to elute the antibody from affinity resins, which certainly cause some damages on the antibody structure and biologic activity. Hydrophobic charge-induction chromatography (HCIC) is also one of new techniques for antibody purification with dual-mode ligands that combine hydrophobic and electrostatic interactions. Target proteins can be adsorbed on uncharged ligands at neutral pH via hydrophobic interactions, and eluted via electrostatic repulsion between target protein and the charged ligand at acidic condition. HCIC process has shown the advantages of salt-tolerance, mild elution and flexible CIP, but the binding selectivity was limited due to relatively simple chemical structure of HCIC ligands. In the present work, peptide ligands were combined with hydrophobic charge-induction groups to developed new type of combinatorial ligands for antibody separation. On one hand, the structure of peptide ligands was designed via the molecular simulation to ensure high affinity to antibody. On the other hand, the hydrophobic charge-induction groups were introduced to enhance the antibody binding and benefit the elution via electrostatic repulsion with the charge-induction effects. The combinatorial ligands were synthesized and coupled onto agarose beads to prepare new affinity resins. The adsorption behaviors of IgG were investigated, and typical pH-dependent adsorption was found. New resins were evaluated with antibody purification from CHO cell culture broth, and high process efficiency was obtained. The results demonstrated that novel combinatorial ligands integrated high affinity of peptide ligand to improve binding selectivity and charge-induction effects for convenient elution, which is promising for cost-effective and large-scale purification of antibodies.

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Liquid chromatographic enantioseparation of (RS)-mexiletine and (RS)-fluoxetine using chiral derivatizing reagents

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Enantiomeric separation of racemic mexiletine and fluoxetine was achieved using three chiral derivatizing reagents (CDRs) based on (S)-naproxen. Diastereomers were synthesized by reaction of mexiletine or fluoxetine with the CDRs and were separated on a C18 column under reversed-phase conditions using a binary mixture of acetonitrile and triethylammonium phosphate/water, with UV detection at 230 and 226 nm. The results obtained for enantioseparation of the two drugs using the three CDRs were compiled and compared. The conditions for derivatization and chromatographic separation were optimized. The method was validated for linearity, repeatability, limit of detection and limit of quantification.

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