

Purification of Radioiodinated Human Insulin by High Performance Liquid Chromatography For A Sensitive Radioimmunoassay

Abstract

The optimal sensitivity of a radioimmunoassay depends on the purity of the radiolabeled antigen. The conventional purification methods are not complete and are time consuming. The combination of a Sep-pak C18 cartridge and high performance liquid chromatography (HPLC) for the purification of ¹²⁵I-labeled insulin in our study revealed that the Sep-pak cartridge can serve as the preliminary step to remove unreacted radioactive iodide, the reactants, and labeled but presumably damaged materials unadsorbed to the cartridge. The fractions eluted from the Sep-pak containing high radioactivity and high immunoreactivity to the antibody were chosen for further purification by HPLC to eliminate undesirable radiolabeled substances with a lesser immunoreactivity. The purified radiolabeled insulin was used to develop a sensitive radioimmunoassay with detecting limits of 0.03 microU/mL per tube.