ABSTRACT

The invention relates to methods of separation and/or purification of impurities yielding a purified heterologous protein product devoid of related impurities or with substantially minimal quantities of such glycosylated impurities. More specifically, the invention relates to the identification of glycosylated forms of insulin analogues such as glargine impurities characterized post expression in yeast based systems such as *Pichia pastoris*. The invention also relates to methods used to clone gene encoding the protein insulin glargine; inserting the related gene in a suitable yeast host; producing culture of the recombinant strain, stimulating expression of the heterologous polypeptide, its secretion and purification post fermentation and related enzymatic conversions.