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Title (English)
Process development for the production of a therapeutic Affibody® Molecule

Title (Swedish)
Processutveckling för att tillverka en Affibody®-molekyl avsedd för cancerterapi

Recently HER3, member of the epidermal growth factor receptor family (EGFR), has been found to play a crucial role in the development of resistance towards inhibitors that are given to patients with HER1- and HER2-driven cancers. As HER3 is up-regulated or over-activated in several types of human cancers, it is of outmost importance that new innovative drugs target its oncologic activity.

The Affibody® Molecule Z08698 inhibits the heregulin induced signalling of HER3 with high affinity (K_D~50 pM). As the Affibody® Molecule is small, has high solubility and outstanding folding kinetics, an effective penetration of tumour tissue is suggested together with a rationalized manufacturing process. Further coupling to an albumin binding domain (ABD) expands the plasma half-life of the molecule, hence increasing the molecule's potential of serving as a therapeutic.

A process development for production of Z08698-VDGS-ABD094 has been established, where the molecule is efficiently produced in the *E. coli* host strain BL21(DE3), through a T7 based expression system. Cultivations were performed with a fed-batch fermentation process and the conditions were further optimized in order to obtain highest expression, while avoiding undesirable modifications like gluconoylations. By employing Design of experiments in combination with multivariate data analysis, a production process resulting in ~3.5 g product/ l culture could be verified. Moreover, thermolysis was evaluated as a suitable method for cell disruption, enabling an easy and cost-effective manufacturing process of the ABD fused Affibody® Molecule.

Keywords
Affibody® Molecule, HER3, EGFR, HER2, cancer therapy, process development, DOE, multivariate data analysis, *E. coli,* ABD, plasma half-life, thermolysis, fed-batch, gluconoylation, BL21(DE3), T7 expression system

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