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(71) Applicant *(for all designated States except US)*: **AVESTHAGEN LIMITED** [IN/IN]; 9th Floor, Discoverer, International Tech Park (ITPB), Whitefield Road, Bangalore 560066, Karnataka (IN).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **PATELL, Villoo Morawala** [IN/IN]; Avesthagen Limited, 9th Floor Discover, International Tech Park(ITPB), Whitefield Road, Bangalore 560 066 (IN). **GUZDER, Sami N.** [IN/IN]; Avesthagen Limited, 9th Floor Discover, International Tech Park(ITPB), Whitefield Road, Bangalore 560 066 (IN). **MAITY, Sunit** [IN/IN]; Avesthagen Limited, 9th Floor Discover, International Tech Park(ITPB), Whitefield Road, Bangalore 560 066 (IN). **SHEKAR, Sunil** [IN/IN]; Avesthagen Limited, 9th Floor Discover, International Tech Park(ITPB), Whitefield Road, Bangalore 560 066 (IN).

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(54) **Title**: A HIGHLY EFFICIENT PROCESS OF PURIFICATION AND PRODUCTION OF RECOMBINANT TRASTUZUMAB

(57) **Abstract**: The present invention relates to use of novel process of production and recovery of recombinant Monoclonal antibody to HER-2, in biologically active form from fluids, includes but not limited to mammalian host cell culture supernatants by performing fermentation and chromatographic procedure separately and jointly. The recovery by the novel process can be very useful in pharmaceutical industries as it as it furnishes the rapid and efficient recovery of Recombinant Trastuzumab.

A Highly Efficient Process Of Purification And Production Of Recombinant Trastuzumab

FIELD OF THE INVENTION

The present invention relates to use of novel process of recovery of Recombinant Trastuzumab (Herceptin), a Monoclonal Antibody to human epidermal growth factor receptor-2 (HER-2), in biologically active form from fluids, includes but not limited to mammalian host cell culture supernatants by performing fermentation and chromatographic procedure separately and jointly. The present invention principally pertains to a novel mode of recovering high yield of purified recombinant Trastuzumab by performing different chromatographic techniques, which can be very useful for the pharmaceutical industries as it furnishes the rapid and efficient recovery of Recombinant Trastuzumab.

BACKGROUND AND PRIOR ART OF THE INVENTION

In past, various media and methods were used for the cell culture manufacturing of recombinant glycoprotein or Monoclonal Antibody. Commonly employed bioreactor process includes; batch, semi fed-batch, fed-batch, perfusion and continuous fermentation. The ever-increasing demand of monoclonal antibody and other recombinant proteins in properly glycosyalted forms have increased the prospects of cell culture process development. In addition the regulatory hurdles imposed on the serum containing process has led to the development of cell culture process in a completely chemically defined environment.

Numerous techniques have in the past been applied in preparative separations of bio-chemically significant materials. Commonly employed preparative separatory techniques include: ultrafiltration, column electrofocusing, flatbed electrofocusing, gel filtration, electrophoresis, isotachophoresis and various forms of chromatography. Among the commonly employed chromatographic techniques are ion exchange and adsorption chromatography. The extensive application of recombinant methodologies to large-scale purification and production of eukaryotic protein has increased the

prospect of obtaining the molecule in required quantity using simplified purification procedures.

Trastuzumab is a recombinant humanized IgG 1k monoclonal antibody glycoprotein that binds specifically to human epidermal growth factor receptor-2 (HER-2). Herceptin is designed to target and block the function of HER-2 protein over expression. The antibody, Trastuzumab contains primarily human constant framework regions with the complementarity–determining regions (CDR) from a murine antibody with specificity for human HER-2. The humanized antibody against HER-2, Trastuzumab having an approximate molecular weight of 155 kilo-daltons is produced by a mammalian cell (Chinese Hamster Ovary) [CHO] suspension culture in a nutrient medium containing the antibiotic gentamicin. Gentamicin is not detectable in the final product. Trastuzumab is packaged as a sterile, white to pale yellow, preservative-free lyophilized powder for intravenous (IV) administration.

Cancerous breast tissue cells that over-express the HER-2 gene produce extra protein receptors on the cell surface. The higher density of receptors triggers the cell to divide and multiply at an accelerated rate, thus contributing to tumour growth. Trastuzumab binds to numerous HER-2 receptor sites found on the cell surface, blocking the receptor sites and possibly preventing further growth by interrupting the growth signal.

US Patent Application number-11/400,638 encompasses Trastuzumab as a single agent is indicated for the treatment of patients with metastatic breast cancer whose tumors over-express the HER-2 protein and who have received one or more chemotherapy regimens for their metastatic disease. Trastuzumab in combination with paclitaxel is indicated for treatment of patients with metastatic breast cancer whose tumors over-express the HER-2 protein and who have not received chemotherapy for their metastatic disease. This antibody should only be used in patients whose tumors have HER-2 protein over-expression.

The present invention principally relates to a new process of recovering higher yield of purified Recombinant Trastuzumab (Herceptin), a Monoclonal Antibody to human epidermal growth factor receptor-2 (HER-2) by performing bio- analytical techniques

which includes but not limited to chromatographic techniques. The recovery by the novel process can be very useful in commercial scale, especially in pharmaceutical industries as it furnishes the rapid and efficient recovery of Recombinant Trastuzumab.

OBJECTIVES OF THE INVENTION

The principal object of the present invention is to use of novel process of production and recovery by performing fermentation and chromatographic procedures for rapid and efficient recovery of recombinant Trastuzumab (Herceptin), a monoclonal antibody to human epidermal growth factor receptor-2 (HER-2), from cell culture supernatant. The present invention leads to a novel process for recovering the maximum yield of the recombinant Trastuzumab (Herceptin).

SUMMARY OF THE INVENTION

The present invention relates to the use of novel process of recovery of Recombinant Trastuzumab (Herceptin), a Monoclonal Antibody to human epidermal growth factor receptor-2 (HER-2) by performing chromatographic procedures, separately and jointly, in biologically active form from fluids, especially mammalian host cell culture supernatants.

The present invention also relates to the use of novel process of recovery by performing fermentation for the over-expression of Recombinant Trastuzumab (Herceptin), a Monoclonal Antibody to human epidermal growth factor receptor-2 (HER-2), protein in Chinese Hamster Ovary (CHO) cells.

DETAILED DESCRIPTION OF THE INVENTION

This present invention relates to the rapid, efficient and effective process of recovery of recombinant Monoclonal antibody to HER-2 from cell culture supernatant from Cell culture fluid by means of chromatography's techniques, especially from Affinity chromatography. Generally, foremost, affinity chromatography is performed for

capture of recombinant Monoclonal antibody. This process of separation involves in selective binding of the desired compound to specific affinity resin and then elution with elution buffer. Supernatants from culture are clarified before treatment of chromatography. Monoclonal Antibody to HER-2 containing eluent fractions are enriched with biologically active material, but they will be subjected to further processing by Ion exchange chromatographic step. These processes are used for removal of process related impurities like host cell protein and host cell DNA. The present invention also relates to the recovery procedure of recombinant Monoclonal Antibody to HER-2 involving serial application of different chromatographic techniques as mentioned previously, which can be really useful in the pharmaceutical preparations while doing antibodies purification. All different steps, conditions and compositions are disclosed in the present invention.

Example 1:

Clarification of the cell culture harvest was carried out by using a cellulose disposable filter with 650 – 1000 cm^2 effective filtration area and with an operating pressure of not more than 30 psi. The filtrate was checked for turbidity and target protein content. Affinity chromatography was used in binding and elution mode with column of 32 mm diameter for capturing; with Tris buffer pH 7.2 – 7.6 as equilibration buffer. After the sample is loaded on to the column, it is washed with equilibration buffer followed by 50 mM Tris-Cl, then with second wash with 50 mM Tris-Cl containing 100-400 mM NaCl pH 7-8 buffer solution. The protein of interest was eluted with citrate buffer pH 3.0-3.8 (Fig 1). The eluate was hold for 45 – 60 min at acidic pH at room temperature for virus inactivation and later neutralized. The Protein A eluate fraction were pooled. Anion exchange chromatography in negative binding mode was carried out at an operational flow rate of 140 cm/hr. The column was equilibrated with Tris buffer pH 6.8 – 7.2. Protein of interest is collected in flow through. This step was used for the removal of process related impurities like leachate protein A, host cell DNA and host cell protein (Fig 2). Thereafter, the flow through was filtered for virus removal using viral removal filter having an effective filtration area of 0.01 m^2. The filtrate was buffer exchanged using a 50-kDa TFF membrane. The buffer used for the diafiltration process is Tris buffer pH 6.8-7.2. Cation exchange chromatography was carried out with the diafiltered protein solution after

equilibrating the column with Tris buffer pH 6.8-7.2. The protein of interest was eluted with elution buffer using NaCl salt gradient. This step was used for the removal of process related impurities like host cell DNA and host cell protein (Fig 3). The eluate was buffer exchanged and concentrated using a 50-kDa TFF membrane at a Trans Membrane Pressure (TMP) of 5-10 psi. The buffer exchanged protein solution was filtered using 0.2μm filter. The drug substance was characterized as per the in house developed specifications. The Drug Substance (Active Pharmaceutical Ingredient) was formulated using the ingredients mentioned below. They are 51 mM Sodium Phosphate buffer pH 6.2, 400 mg α, α-trehalose dihydrate, 9.9 mg L-histidine HCl, 6.4 mg L-histidine, and 1.8 mg polysorbate 20, USP, yields a solution containing 20-mg/mL trastuzumab.

Example 2:

The formulated material was characterized as per the specifications set by product development specification so as to meet the physico-chemical parameter comparable with the originator. A 10% Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis (SDS PAGE) under reducing condition was studied for the sample derived from the PAGE showed a clear corresponding band with Reference Medical Product (RMP) reflecting the fact that there is a comparable purity almost to homogeneity and integrity in terms of stability (Fig 4). Protein A High Performance Liquid Chromatography (HPLC) profile carried out during this step with test molecule showed a clear single peak, as its retention time is very much comparable with that of the RMP. It also suggests that the test molecule in question is very specific and pure with respect to the RMP. This technique is used as one of the orthogonal methods for estimation of target protein (Fig 5). Ion exchange chromatography HPLC profile showed for test molecule, which was very much comparable with the RMP. The charge variants profile indicates the similar variants in the RMP. This is one analytical tool to determine the charge variant exists in a molecule (Fig 6). Size exclusion chromatography reveals the approximate molecular weight, hydrodynamic radius and also the determination of oligomeric status. SEZ showed for test molecule, which was very much comparable with the RMP with respect to retention time where in confirms the size in terms of the mass is same with that of the originator (Fig 7).

CLAIMS

We claim:

1. A process for the recovery and purification of recombinant Monoclonal antibody to HER2 comprising steps of: contacting culture supernatant(s) with resin(s) for selective adsorption of compound(s); eluting the adsorbed compound and subjecting the enriched product to series of separation techniques.
2. The process as claimed in claim 1, where elution of adsorbed compound is performed by affinity chromatography.
3. The process as claimed in claim 1, where monoclonal antibody to HER2 containing eluent fractions are purified using ion exchange chromatography.
4. The process as claimed in claim 1, wherein supernatant is obtained from cell fermentation.
5. The process as claimed in claim 1, wherein said supernatant is mammalian host cell culture supernatant.
6. The process as claimed in claim 1, wherein said supernatant is cell culture-derived fluid.
7. The process as claimed in claim 1, wherein said supernatant is mammalian cell culture derived fluid
8. The process as claimed in claim 1, wherein the process buffer 20-100 mM Tris buffer pH 6.4-7.2 with 100-400 mM NaCl is used for the recovery of target protein from culture supernatant.
9. The process as claimed in claim 1, wherein the process buffer is particularly 40-60 mM Tris buffer pH 6.6-7.0 with 200-300mM NaCl is used for the recovery of target protein from culture supernatant.
10. The process as claimed in claim 1, wherein said culture supernatant(s) are concentrated and clarified before contacting resins.
11. The process as claimed in claim 1, where eluent is held for 45 – 60 min at acidic pH at room temperature for virus inactivation.
12. The process as claimed in claim 1, where recombinant Monoclonal antibody to HER2recovered and purified is Trastuzumab.

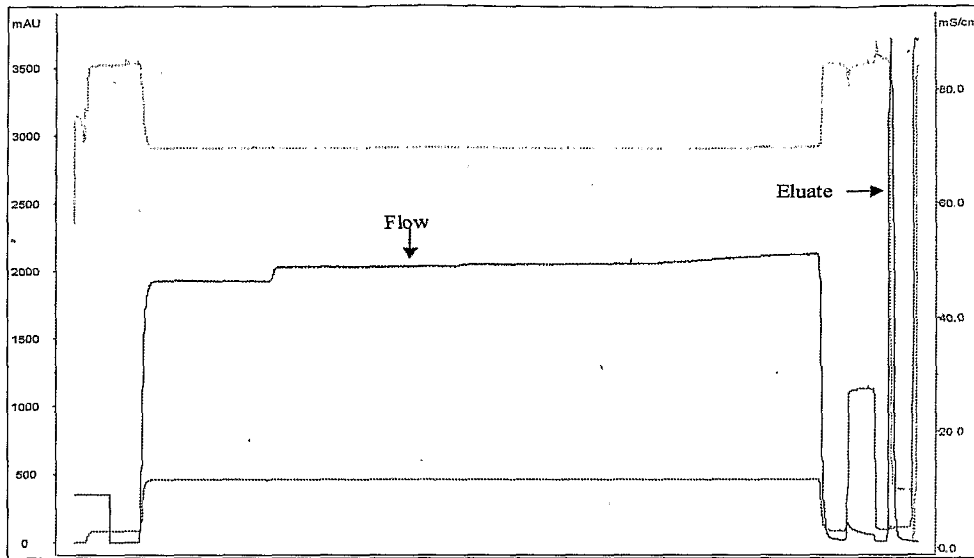


Figure 1: Process chromatogram of affinity chromatography

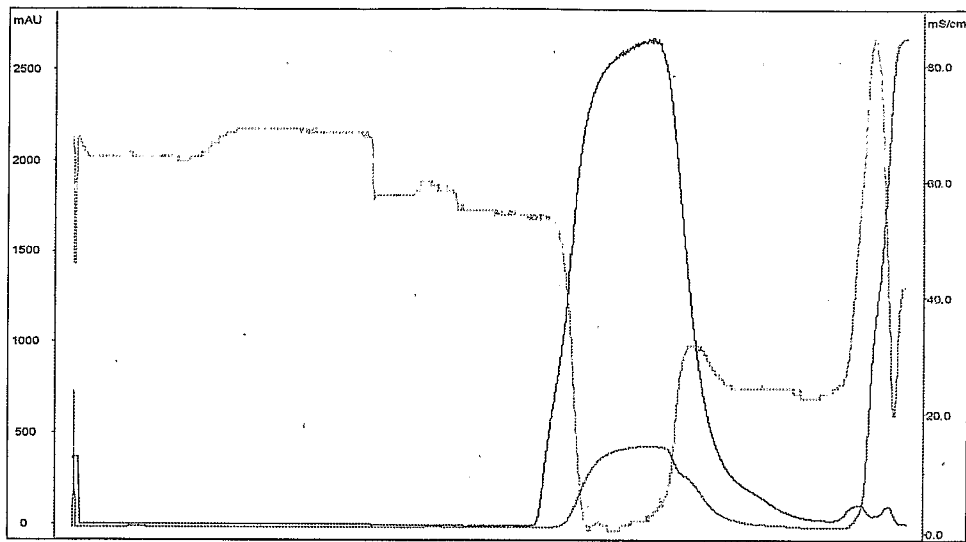


Figure 2: Process chromatogram of anion chromatography

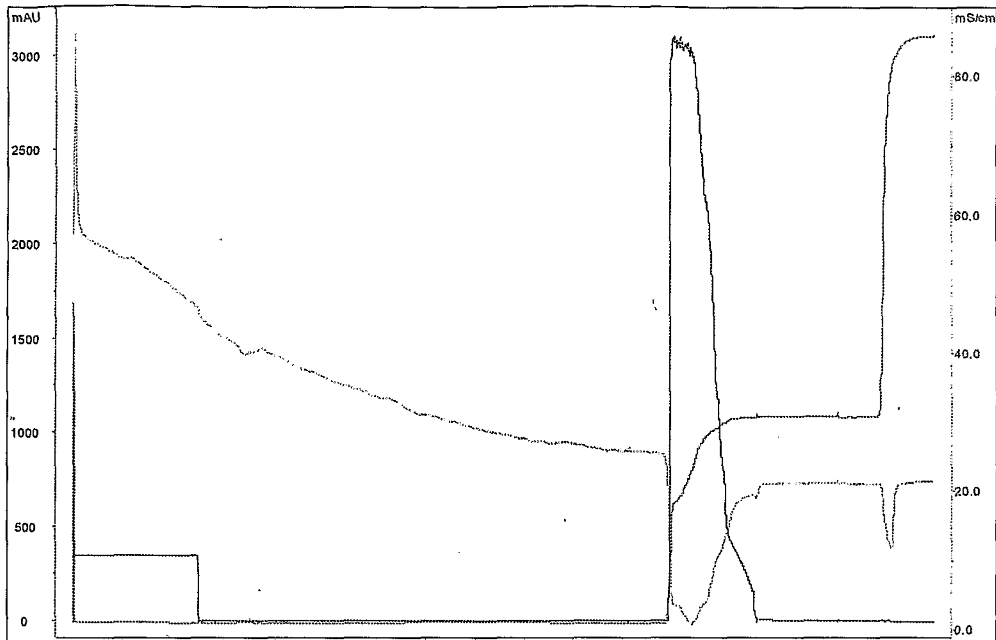


Figure 3: Process chromatogram of cation chromatography

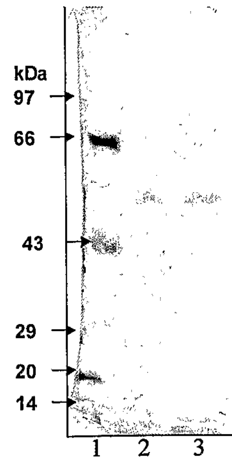
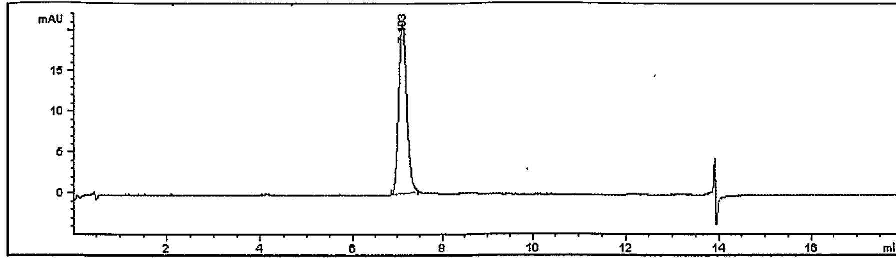


Figure 4: Electrophoretic pattern of Drug substance

RMP:



Trastuzumab:

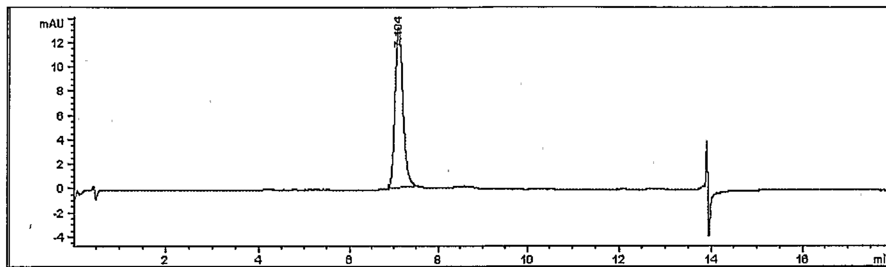
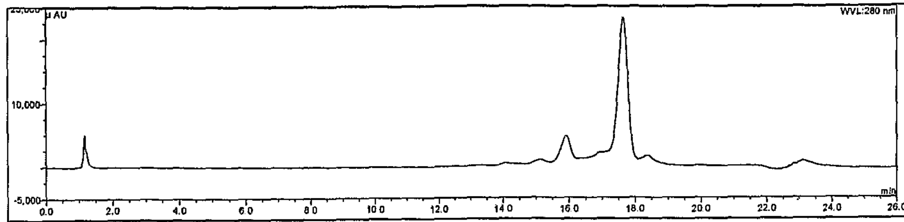


Figure 5: Protein A HPLC profile of Drug substance showing comparable profile with RMP

RMP:



Trastuzumab:

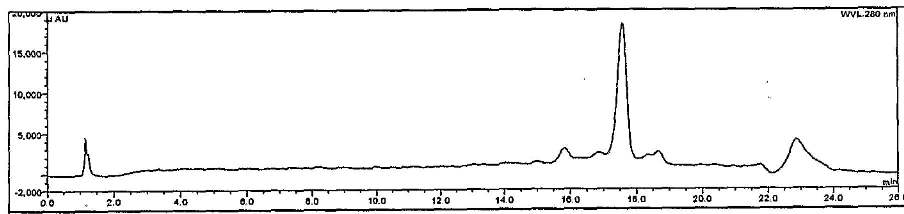
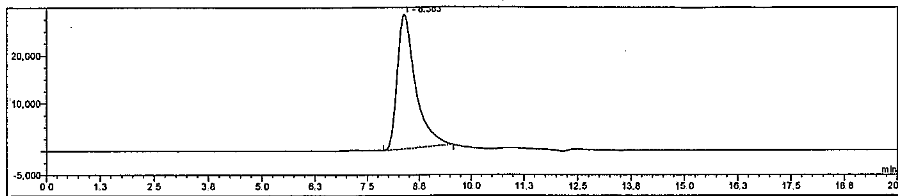


Figure 6: IEX HPLC profile of Drug substance showing comparable profile with RMP

RMP:



Trastuzumab:

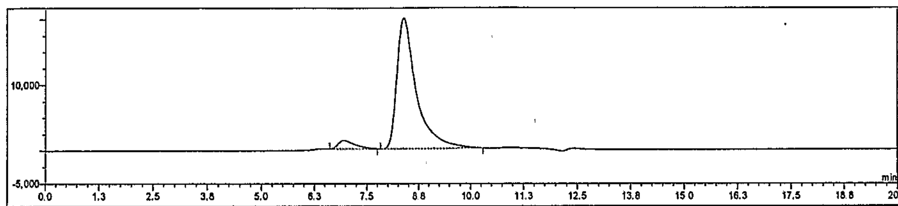


Fig 7: SEC HPLC profile of Drug substance showing comparable profile with RMP