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(54) Title: A COMPOSITION COMPRISING ANTIBODY WITH REDUCED LEVEL OF BASIC VARIANTS THEREOF

(57) Abstract: The present invention relates to a composition of an antibody, preferably pertuzumab wherein the said composition comprises reduced level of antibody variants, preferably basic variants of the antibody. More preferably, the said composition comprises no more than 1 % of antibody variants containing signal peptide residue(s). Pertuzumab is a preferred antibody according to the present invention. The present invention also provides a method of making such composition.

A COMPOSITION COMPRISING ANTIBODY WITH REDUCED LEVEL OF BASIC VARIANTS THEREOF

FIELD OF THE INVENTION

The present invention relates to a composition of an antibody, preferably pertuzumab wherein the said composition comprises reduced level of antibody variants, preferably the basic variants of the pertuzumab. More preferably, the said composition comprises no more than 1 % of antibody variants containing signal peptide residue(s). The present invention also provides a method of making such composition.

BACKGROUND OF THE INVENTION

The continuous increase in the number of approved monoclonal antibody (mAb) based therapy suggests that mAbs, and their derivatives, will continue to be the focus of the biotherapeutics industry for years to come. Although vast improvements in capability to manufacture, characterize, and stabilize mAbs have been achieved, there are still challenges to overcome which include analytical and stabilization approaches associated with the development of mAb compositions. As we know, biological activity of mAb-based therapeutics is closely tied to their structural, conformational and chemical stability, therefore it is always preferable to have composition of antibody with less structural, conformational and post-translational modified variants.

Present invention focuses on one of such known challenges related to preparation of antibody composition. The present invention provides pertuzumab composition wherein composition has less than 1 % of at least one of the basic variants containing signal peptide residue(s). It also provides a method to prepare pertuzumab composition wherein composition has less than 1 % of at least one of the basic variants containing signal peptide residue(s).

Pertuzumab is a recombinant humanized monoclonal antibody that targets the extracellular dimerization domain (Subdomain II) of the human epidermal growth factor receptor 2 protein (HER2). Pertuzumab is produced by recombinant DNA technology in a mammalian cell (Chinese Hamster Ovary). Method of making pertuzumab molecule is described in patent document WO 2001/000245 published on 04th January, 2001.

Patent document EP1771482B1 discloses composition comprising pertuzumab and 5 % to

about 15 % of the pertuzumab product related variant (pertuzumab variant) wherein pertuzumab variant has VHS- amino acid residues on N-terminal of one or two light-chain component(s) of pertuzumab. This variant is referred herein as VHS- variant of pertuzumab. The said VHS- variant is an antibody variant wherein antibody has VHS- amino acid residues on N-terminal region of its one or two light-chain component(s) resulted from incomplete cleavage of the signal peptide. Since VHS- portion does not represent active pertuzumab, this pertuzumab variant containing VHS- residues is an undesired variant of pertuzumab. Thus, it is always preferable to have composition of pertuzumab without such variant. Therefore, present invention provides novel and improved composition of pertuzumab wherein the said composition has less than 1 % of at least one of the basic variants containing signal peptide residue(s).

SUMMARY OF THE INVENTION

The present invention is related to a composition of antibody wherein the composition is free from antibody variant containing signal peptide residue(s). Preferably, the present invention provides pertuzumab composition wherein the said composition has less than 1 % of at least one of the basic variants containing signal peptide residue(s). According to the present invention, an antibody variants containing signal peptide residue(s) is VHS- variant as described, herein. Also, the present invention provides method of making the said composition using suitable purification technique(s). The pertuzumab composition according to the present invention can be used for therapeutic purpose either in single dose or multi-dose form.

BRIEF DESCRIPTION OF THE ACCOMPANYING FIGURES

Figure 1 shows the charge variant profile of the Multi-modal column purified pertuzumab by analytical HP-ion exchange chromatography (HP-IEC). The figure shows that VHS- variant of pertuzumab and other variants eluted after VHS- variant peak were no more than 0.5 %.

Figure 2 shows the charge variants profile of the SCEX column purified pertuzumab by analytical HP-ion exchange chromatography (HP-IEC). The figure shows that VHS- variant and other variants eluted after VHS- variant peak were no more than 0.5 %.

Figure 3 shows the charge variants profile of the multi-modal column purified pertuzumab by analytical HP-ion exchange chromatography (HP-IEC) after CpB treatment. The figure shows

basic variants profile of pertuzumab sample before (load) and after (eluted) purification by multi-modal column. Figure 3 clearly shows that purified composition of pertuzumab obtained after multi-modal chromatography has VHS- variant (one of the basic variants) no more than 0.5 %.

Figure 4 shows the charge variants profile of the strong cation exchange (SCEX) column purified pertuzumab by analytical HP-ion exchange chromatography (HP-IEC) after CpB treatment. The figure shows basic variants profile of a pertuzumab sample before (load) and after (eluted; Fr 1) purification by SCEX column. Figure 4 clearly shows that purified composition of pertuzumab obtained after SCEX column chromatography has VHS- variant (one of the basic variants) no more than 0.5 %.

The abbreviations used in the present application specific are defined below:

ADCC: Antibody dependent cell mediated cytotoxicity

CpB: Carboxypeptidase B

DF: Diafiltration

DNA: Deoxyribonucleic acid

Fr 1: Fraction 1

Fr 2: Fraction 2

HCP: Host cell protein

HIC: Hydrophobic interaction chromatography

HP-IEC: High performance ion exchange chromatography

HP-SEC: High performance size exclusion chromatography

mAbs: Monoclonal antibodies

Mixed mode column: Hydrophobic interaction and anion exchange column chromatography

Multi-modal column: Strong cation, weak cation and strong anion column chromatography

MWCO: Molecular weight cut-off

NaCl: Sodium chloride

Protein A: Protein A cross-linked agarose column

r-PA: recombinant Protein A

SCEX: Strong cation exchange column chromatography

UF: Ultrafiltration

VHS- : Valine-Histidine-Serine-

WFI: Water for Injection

EMBODIMENTS OF THE INVENTION

In one embodiment, the present invention provides a composition of antibody with less heterogeneity. In a preferred aspect, the antibody is an anti-HER2 antibody. In a more preferred aspect, the anti-HER2 antibody is pertuzumab.

In a preferred embodiment, the present invention provides a composition of pertuzumab with less heterogeneity.

In a second embodiment, the present invention provides a composition of antibody wherein composition comprises an antibody and no more than 1 % of antibody variants containing signal peptide residue(s), preferably no more than 0.5 % of the antibody variants containing signal peptide residue(s), more preferably no more than 0.2 % of the antibody variants containing signal peptide residue(s).

In a third embodiment, the present invention provides a composition of antibody wherein the composition comprises an antibody and reduced level of the basic variants.

In a preferred embodiment, the present invention provides a composition of pertuzumab wherein composition comprises pertuzumab and reduced level of the pertuzumab basic variants.

In further embodiment, the present invention provides a composition of pertuzumab wherein the composition comprises pertuzumab and no more than 10 % of pertuzumab basic variants, preferably no more than 7 % of pertuzumab basic variants.

In a fourth embodiment, the present invention provides a composition of antibody wherein composition comprises an antibody and less than 1 % of at least one of the basic variants containing signal peptide residue(s) of the said antibody, preferably less than 0.5 % of at least one of the basic variants containing signal peptide residue(s) of the said antibody, more preferably less than 0.2 % of at least one of the basic variants containing signal peptide residue(s) of the said antibody.

In a preferred embodiment, the present invention provides a composition of pertuzumab wherein composition comprises pertuzumab and less than 1 % of at least one of the basic variants containing signal peptide residue(s) of pertuzumab, preferably less than 0.5 % of at

least one of the basic variants containing signal peptide residue(s) of pertuzumab, more preferably less than 0.2 % of at least one of the basic variants containing signal peptide residue(s) of pertuzumab.

In a fifth embodiment, the present invention provides a composition of antibody wherein composition is free from antibody variant containing signal peptide residue(s).

In a preferred embodiment, the present invention provides a composition of pertuzumab wherein composition is free from pertuzumab variant containing signal peptide residue(s).

In a sixth embodiment, the present invention provides a composition of antibody wherein composition has VHS- variant no more than 1 %, preferably no more than 0.5 %, more preferably no more than 0.2 %.

In preferred embodiment, the present invention provides a composition of pertuzumab wherein composition has VHS- variant no more than 1 %, preferably no more than 0.5 %, more preferably no more than 0.2 %.

In seventh embodiment, the present invention provides a composition of antibody wherein composition is free from VHS- variant.

In a preferred embodiment, the present invention provides a composition of pertuzumab wherein composition is free from VHS- variant.

In a further embodiment, the present invention provides a composition of antibody wherein an amount of aggregates in composition of antibody is no greater than 1 %, preferably no greater than 0.5 %.

In a preferred embodiment, the present invention provides a composition of pertuzumab wherein an amount of aggregates in composition of pertuzumab is no greater than 1 %, preferably no greater than 0.5 %.

In one more embodiment, the composition of antibody according to the present invention provides anti-proliferative activity.

In a preferred embodiment, the composition of pertuzumab according to the present invention provides anti-proliferative activity.

In one more embodiment, the composition of antibody according to the present invention provides ADCC activity.

In preferred embodiment, the composition of pertuzumab according to the present invention provides ADCC activity.

In eighth embodiment, the present invention provides method of making a composition of antibody wherein the composition has no more than 1 % of antibody variants containing signal peptide residue(s), preferably no more than 0.5 % of antibody variants containing signal peptide residue(s), more preferably no more than 0.2 % of antibody variants containing signal peptide residue(s).

In ninth embodiment, the present invention provides method of making a composition of antibody wherein the composition comprises antibody and reduced level of basic variants.

In a preferred embodiment, the present invention provides method of making a composition of pertuzumab wherein the composition comprises pertuzumab and reduced level of pertuzumab basic variants.

In further embodiment, the present invention provides method of making a composition of pertuzumab wherein the composition comprises pertuzumab and no more than 10 % of pertuzumab basic variants, preferably no more than 7 % of pertuzumab basic variants.

In tenth embodiment, the present invention provides method of making a composition of antibody wherein composition has less than 1 % of at least one of the basic variants containing signal peptide residue(s) of the antibody, preferably less than 0.5 % of at least one of the basic variants containing signal peptide residue(s) of the antibody, more preferably less than 0.2 % of at least one of the basic variants containing signal peptide residue(s) of the antibody.

In a preferred embodiment, the present invention provides method of making a composition of pertuzumab wherein composition has less than 1 % of at least one of the basic variants containing signal peptide residue(s) of pertuzumab, preferably less than 0.5 % of at least one of the basic variants containing signal peptide residue(s) of pertuzumab, more preferably less than 0.2 % of at least one of the basic variants containing signal peptide residue(s) of pertuzumab.

In eleventh embodiment, the present invention provides method of making a composition of pertuzumab wherein composition is free from VHS- variant or the composition has VHS- variant no more than 1 %, preferably no more than 0.5 %., more preferably no more than 0.2 %.

In a further embodiment, the composition of antibody according to present invention is obtained by suitable purification steps wherein the purification method comprises one or more column chromatography and membrane ultrafiltration-diafiltration.

In a preferred embodiment, the composition of pertuzumab according to the present invention is obtained by suitable purification steps wherein the purification method comprises one or more chromatography and membrane ultrafiltration-diafiltration.

In a furthermore embodiment, the chromatography used may be selected from affinity chromatography, cation exchange chromatography, mixed mode chromatography, multi-modal chromatography and/or combination thereof along with membrane ultrafiltration-diafiltration.

In one more embodiment, the composition of antibody according to present invention is obtained when purified by a method comprising following steps:

a) Affinity chromatography preferably, protein A chromatography
b) Cation exchange chromatography or multi-modal chromatography optionally, followed by other purification method such as chromatography method, UF/DF, microfiltration, and combination thereof. Further chromatography method includes, but not limited to, anion exchange chromatography, cation exchange chromatography, HIC, mixed mode chromatography and the like.

In a preferred embodiment, the composition of antibody according to present invention is obtained through purification by a method comprising following steps:

a) Affinity chromatography, preferably, protein A chromatography
b) Cation exchange chromatography or multi-modal optionally, followed by
c) Mixed mode chromatography

In more preferred embodiment, the composition of antibody according to present invention in obtained by the purification steps sequentially carried out as follows:

a) Affinity chromatography, preferably, protein A chromatography
b) Low-pH incubation
c) Neutralization and reconditioning by UF/DF
d) Cation exchange chromatography or multi-modal chromatography
e) Ultrafiltration-diafiltration
f) Mixed mode chromatography

Preferably, according to the present invention pertuzumab is prepared using purification process as described herein above.

In one embodiment, the present invention provides method of making composition of antibody wherein an amount of aggregates in composition of antibody is no greater than 1 %, preferably no greater than 0.5 %.

In preferred embodiment, the present invention provides method of making composition of pertuzumab wherein an amount of aggregates in composition of pertuzumab is no greater than 1 %, preferably no greater than 0.5 %.

DETAILED DESCRIPTION OF THE INVENTION

Definitions of the terms used herein the present invention:

An "amino acid sequence variant" of antibody according to the present invention includes substitutions, deletions, and/or additions at certain positions within or adjacent to the amino acid sequence of the main antibody. Examples of amino acid sequence variants include acidic variants (e.g. deamidated antibody variants), basic variants, (e.g. the antibody with an amino-terminal signal peptide extension, VHS- on one or two light-chain components thereof, antibody with a C-terminal lysine residue on one or two heavy-chain components) thereof, etc. and a combination of variations to the amino acid sequences of the heavy- and/or light-chain components. The antibody variant of particular interest herein is the antibody comprising an amino-terminal signal peptide extension on one or two light chains thereof.

As used herein, the term "diafiltration step" refers to a total volume exchange during the process of diafiltration.

As used herein, the term "diafiltration" or "DF" is used to mean a specialized class of filtration in which the retentate is diluted with solvent and re-filtered, to reduce the concentration of soluble permeates components. Diafiltration may or may not lead to an increase in the concentration of retained components, including, for example, antibodies, when performed with constant-volume mode. For example, in continuous diafiltration, a solvent is continuously added to the retentate at the same rate as the filtrate is generated. In this case, the retentate volume and the concentration of retained components mainly the antibody do not change during the process. On the other hand, in discontinuous or sequential dilution diafiltration, an ultrafiltration step is followed by the addition of solvent to the retentate side; if the volume of solvent added to the retentate side is not equal or greater to the volume of filtrate generated, then the retained components will have a high concentration.

Diafiltration may be used to alter the pH, ionic strength, salt composition, buffer composition, or other properties of a solution or suspension of macromolecules.

As used herein, the term "mixed mode column chromatography" or "mixed mode chromatography" refers to chromatographic method that utilize at least two different forms of interaction between the stationary phase and analytes in order to achieve their separation. Preferably, mixed mode column or membrane comprises two different types of resins. Preferably, the mixed mode column comprising hydrophobic interaction resin and anion exchange resin are used in the present invention. The term "analytes" used herein is known to a skilled person and it is referred as the substances to be separated during chromatography.

As used herein, the term "multi-modal column chromatography" or "multi-modal chromatography" refers to chromatographic method that utilize at least more than two different forms of interaction between the stationary phase and analytes in order to achieve their separation. Preferably, the multi-modal column comprising strong cation exchange resin, weak cation exchange resin and strong anion exchange resin are used in the present invention.

As used herein, the terms "ultrafiltration" or "UF" refers to any technique in which a solution or a suspension is subjected to a semi-permeable membrane that retains macromolecules while allowing solvent and small solute molecules to pass through. Ultrafiltration may be used to increase the concentration of macromolecules in a solution or suspension. In a preferred embodiment, ultrafiltration is used to increase the concentration of an antibody in the desired media.

As used herein, the terms "ultrafiltration-diafiltration" or "UF/DF" refers to any process, technique or combination of techniques that accomplishes ultrafiltration and/or diafiltration, either sequentially or simultaneously.

The term "An antibody variant containing signal peptide residue(s)" or "signal peptide residual variant" or "leader extension" or "at least one of the basic variants containing signal peptide residue(s)" as described herein is an antibody variant wherein few amino acid residue(s) of signal peptide or leader sequence are present on heavy- and/or light-chain components due to incomplete cleavage of signal peptide. This variant is one of the product related variants. If such variant has basic pI value, its elution is expected to occur after the main species of antibody when analyzed by a CEX column and therefore it can be identified as post-principal peak in the CEX chromatogram. If such variant has acidic pI value, its

elution is expected to occur before the main species of antibody and therefore it can be identified as pre-principal peak in the CEX chromatogram.

The term "antibody" as referred to herein includes whole antibodies and any antigen-binding fragment (i.e., "antigen-binding portion") or single chains thereof. An "antibody" refers to a glycoprotein comprising at least two heavy (H) chains and two light (L) chains interconnected by disulphide bonds, or an antigen-binding portion thereof. Each heavy chain is comprised of a heavy chain variable region (abbreviated herein as VH) and a heavy chain constant region. The heavy chain constant region is comprised of three domains, CH1, CH2 and CH3. Each light chain is comprised of a light chain variable region (abbreviated herein as VL) and a light chain constant region. The light chain constant region is comprised of one domain, CL. The VH and VL regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each VH and VL is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FRl, CDRl, FR2, CDR2, FR3, CDR3, FR4. The variable regions of the heavy and light chains contain a binding domain that interacts with an antigen. The constant regions of the antibodies may mediate the binding of the immunoglobulin to host tissues or factors, including various cells of the immune system (e.g., effector cells) and the first component (C1q) of the classical complement system. Pertuzumab is an antibody according to the present invention. Therefore, pertuzumab as referred herein includes amino acid sequences of full-length heavy and full-length light chains or variable regions of heavy and light chains of pertuzumab or antigen binding portion of pertuzumab.

The term "basic variant" according to the present invention is an antibody variant which represents a different charge on the surface with a pI value higher than the pI value of main species. VHS- variant as described herein is a type of basic variants.

The term "bind-elute mode" refers to an operational approach to chromatography in which the buffer conditions are established so that the desired protein and some product related substances bind to the column upon application, which are eluted differentially by with modified buffer conditions and collected in fractions. Fractionation is most commonly achieved by applying an elution gradient, in which the concentration of one or more buffer components, or conditions such as pH, are increased or decreased. The increase or decrease

may be essentially continuous, as in the case of so-called linear gradients, or incremental, as in the case of so-called step gradients.

The term "clarified" refers to a sample (i.e. a cell suspension) having undergone a solid-liquid separation step involving one or more of centrifugation, microfiltration and depth filtration to remove host cells and/or cellular debris. A clarified fermentation broth may be a cell culture supernatant. Clarification is sometimes referred to as a primary or initial recovery step and typically occurs prior to any chromatography or a similar step. Present invention provides a composition of antibody with less heterogeneity. Composition of antibody with less heterogeneity according to the present invention comprises reduced level of specific pertuzumab variant as compared to known amount of the same variant. Present invention provides a composition of antibody with reduced level of basic variants. Preferably, the present invention provides a composition of pertuzumab with reduced level of basic variants of pertuzumab. Said basic variants can be isolated and identified by HP-IEC and mass spectrometry.

The term "flow-through-and-wash mode" refers to an operational approach to chromatography in which the buffer conditions are established so that the desired protein flows through the column upon application while undesired substances remain bound to matrix or retained by the matrix, thus achieving their removal in the subsequent steps.

The term "heterogeneity" described herein is defined as a phenomenon wherein secreted antibodies have various forms. Such heterogeneity may result from differential charge or size or hydrophobicity variants. It can be but not limited to an amino acid sequence variant. During manufacture of mAbs, various forms of microheterogeneity in size, charge and other parameters occur due to enzymatic processes or spontaneous degradation and modifications during and after protein translation. mAbs undergo chemical degradation via several different mechanisms, including oxidation, deamidation, isomerization and fragmentation, that result in the formation of various charge variants and heterogeneity, thus modifying their isoelectric pH (pI) values (MAbs. 2010 Nov-Dec; 2(6): 613–624). The said types of heterogeneity including charge heterogeneity is known to the skilled person. Such heterogeneity leads to generation of variety of antibody variants of main species antibody as illustrated here.

The term "less heterogeneity" according to the present invention refers to an amount of specific antibody variant that is less as compared to known amount of the same variant.

Preferably, antibody composition comprising reduced level of basic variants, more preferably reduced or no VHS- variant. Known amount can be referred to the amount of specific variant present in unpurified or partially purified sample or antibody composition or antibody mixture. In some instances, it can also refer to the amount of specific antibody variant known in the art.

The term "reduced level" as referred herein depicts level of basic variants present in pertuzumab composition of the present invention as compared to basic variants present in the reference product. Pertuzumab composition according to the present invention comprises basic variants no more than 10 %. It can also be defined as a level of basic variant(s) which are less in quantity as measured by percentage (%) unit after purification step as compared to quantity of the same variant present before the column purification. The reduced level of basic variants is shown here by chromatogram of load (before column purification) and elute (after column purification) of various chromatography steps.

The term "sequential" or "sequentially" refers to a specific order of purification steps with or without intermediates step(s). Intermediate step(s) referred herein are the step(s) required to tune up purified sample solution obtained from the previous step with the subsequent purification step.

The term "signal peptide" as described herein is a peptide (sometimes referred to as signal sequence, targeting signal, localization signal, localization sequence, transit peptide, leader sequence or leader peptide) is a short peptide (usually 16-30 amino acids long) present at the N-terminus of the newly synthesized proteins that are destined towards the secretory pathway.

The term "VHS- variant" as described herein is an antibody variant wherein antibody has VHS- amino acid residues (valine-histidine-serine- residues) on N-terminal region of its one or two light-chain and/or heavy-chain component(s) resulted from incomplete cleavage of the signal peptide containing VHS- residues at C-terminal end of signal peptide. This variant is one of product related variants. It is also known as amino-terminal leader extension (patent document EP1771482B1). VHS- containing antibody molecule has basic pI value and therefore, its elution takes place following the elution of main species antibody by cation exchange chromatography or multi-modal chromatography. The VHS- variant can substantially be isolated by performing HP-IEC and identified by mass spectrometry. Herein the current application, VHS- variant was checked by HP-IEC for pertuzumab sample before

as well as after CpB treatment. It is known to the skilled person that CpB treatment to the antibody sample leads to inclusion of Lys1 and Lys2 variants in main peak and therefore, peaks of Lys1 and Lys2 variants generally do not appear in HP-IEC for CpB treated antibody sample.

The term "partially purified" refers to a mixture or a composition or a sample that has already been subjected to at least one chromatography or other purification step, e.g., non-affinity chromatography, affinity chromatography, etc.

The terms "ultrafiltration-diafiltration" and "membrane ultrafiltration-diafiltration" can be used interchangeably according to the present invention.

The term "about" refers to variation in the numerical quantity that can occur, for example, through typical measuring and handling procedures used for making compounds, compositions, concentrates or formulations; through inadvertent error in these procedures; through differences in the manufacture, source, or purity of starting materials or ingredients used to carry out the methods, and other similar considerations. The term "about" also encompasses amounts that differ due to aging of a composition with a particular initial concentration or mixture, and amounts that differ due to mixing or processing a formulation with a particular initial concentration or mixture.

In one of the aspects, the present invention provides a composition of antibody comprising an antibody and an amount of antibody variant no greater than 1 %. Preferably composition comprises an amount of antibody variant no greater than 0.5 %. More preferably composition comprises an amount of antibody variant no greater than 0.2 %. The amount of antibody variant according to present disclosure include each integer and non-integer number given in particular ranges. For Example, amount of antibody variant in antibody composition is no greater than 1 %, no greater than 0.9 %, no greater than 0.8 %, no greater than 0.7 %, no greater than 0.6 %, no greater than 0.5 %, no greater than 0.4 %, no greater than 0.3 %, no greater than 0.2 % or no greater than 0.1 %.

The present invention provides a composition of antibody wherein antibody composition is free from antibody variant containing signal peptide residue(s). Preferably, the present invention provides a composition of pertuzumab wherein composition is free from pertuzumab variant containing signal peptide residue(s). Pertuzumab variant containing signal peptide residue(s) according to the present invention is a pertuzumab variant wherein few

amino acid residue(s) of signal peptide are present on heavy- and/or light-chain component(s) due to alternate or incomplete cleavage of signal peptide.

In one of the aspects, the present invention provides a composition comprising an antibody and reduced level of the basic variants. Preferably, the present invention provides a composition comprising pertuzumab and reduced level of pertuzumab basic variants.

In one aspect, the present invention provides a composition comprising pertuzumab and no more than 10 % of pertuzumab basic variants, preferably no more than 7 % of pertuzumab basic variants. The amount of basic variants according to present disclosure include each integer and non-integer number given in particular ranges. For Example, amount of pertuzumab basic variants in composition is no more than 10 %, no more than 9 %, no more than 8 %, no more than 7 %, no more than 6.5 %, no more than 6 %, no more than 5.8 %, no more than 5 %, no more than 4 %, no more than 3 %, no more than 2 % or no more than 1 %.

In one of the aspects, the present invention provides a composition of antibody comprising an antibody and an amount of VHS- variant no greater than 1 %. Preferably, composition comprises an amount of VHS- variant no greater than 0.5 %. More preferably composition comprises an amount of VHS- variant no greater than 0.2 %. The amount of VHS- variants according to present disclosure include each integer and non-integer number given in particular ranges. For example, the invention provides a composition of antibody comprising an antibody and an amount of VHS- variant wherein an amount of VHS- variant is no greater than 1 %, no greater than 0.9 %, no greater than 0.8 %, no greater than 0.7 %, no greater than 0.6 %, no greater than 0.5 %, no greater than 0.4 %, no greater than 0.3 %, no greater than 0.2 %, no greater than 0.1 %, no greater than 0.05 % or no greater than 0.03 %.

In one of the aspects, the present invention provides a composition of antibody comprising an antibody and reduced level of basic variants as analyzed by HP-IEC. Preferably, the present invention provides a composition of pertuzumab comprising pertuzumab and reduced level of pertuzumab basic variants as analyzed by HP-IEC.

In one of the aspects, the present invention provides a composition of antibody comprising an antibody and an amount of VHS- variant no greater than 1 % as analyzed by HP-IEC. Preferably, composition comprises an amount of VHS- variant no greater than 0.5 % as analyzed by HP-IEC. More preferably composition comprises an amount of VHS- variant no greater than 0.2 % as analyzed by HP-IEC. For example, the invention provides a composition

of antibody comprising an antibody and an amount of VHS- variant wherein an amount of VHS- variant is no greater than 1 %, no greater than 0.9 %, no greater than 0.8 %, no greater than 0.7 %, no greater than 0.6 %, no greater than 0.5 %, no greater than 0.4 %, no greater than 0.3 %, no greater than 0.2 %, no greater than 0.1 %, no greater than 0.05 % or no greater than 0.03 %, as analyzed by HP-IEC.

The term "percentage" or "%" referred herein is with respect to total chromatographic peak area of the antibody preparation resolved by HP-IEC. For example, VHS- variant no greater than 1 % means VHS- variant is present in 1 % or less than 1 % of the total charge variants profile of antibody preparation as obtained by HP-IEC.

The term "no more than" or "no greater than" or "less than", according to the present invention, can be used interchangeably.

An antibody according to the present invention is a monoclonal antibody. Monoclonal antibody according to present invention is an antibody of which amino acid sequence of heavy-chain or light-chain component is expressed using signal peptide where terminal residues of signal peptide is VHS-. Monoclonal antibodies according to the present invention can be selected from anti-HER2 antibody, anti-CD20 antibody, anti-PD1 antibody, anti-HER3 antibody, anti-EGFR antibody, anti-VEGF antibody, anti-PDL1 antibody, anti-IL13 antibody, anti-IL17 antibody, anti-CD40 antibody, anti-CD37 antibody, anti-LAG3 antibody, anti-C5 antibody, anti-C3 antibody, anti-OX40 antibody, ant-TNF antibody, anti-IL6 antibody, anti-IL4 antibody, anti-IL5 antibody, anti-TNFR antibody, anti-properdin antibody, anti-factor D antibody, anti-factor B antibody, anti-IL2 antibody, anti-CTLA-4 antibody. The anti-HER2 antibody according to the present invention can be selected from ertumaxomab, gancotamab, margetuximab, pertuzumab, timigutuzumab, trastuzumab, trastuzumab beta, zenocutuzumab and the like. Other monoclonal antibodies according to the present invention can be selected from pertuzumab, rituximab, trastuzumab, bevacizumab, nivolumab, pembrolizumab, omalizumab, olartumab, adalimumab, ustekinumab, tocilizumab, bimekizumab, ristekinumab, ixexizumab, infliximab, brodalumab, dupilumab, ocrelizumab, mepolizumab, daratumumab, secukinumab, atezolizumab, evolocumab, alirocumab, denosumab, canakinumab, certolizumab, natalizumab, almetuzumab, obinutuzumab. More preferably, the monoclonal antibody according to the present invention is pertuzumab or trastuzumab.

More preferably, the present invention provides a composition of pertuzumab wherein composition is free from VHS- variant of pertuzumab. VHS- variant of pertuzumab described as herein is a variant wherein VHS- residues of signal peptide are present on heavy- and/or light-chain component of pertuzumab.

In one of the aspects, the present invention provides a composition of pertuzumab wherein composition is free from VHS- variant of pertuzumab as analysed by HP-IEC. VHS- variant of pertuzumab described as herein is a variant wherein VHS- residues of signal peptide are present on heavy- and/or light-chain component of pertuzumab.

In a further aspect, the present invention provides a composition of antibody wherein the amount of aggregates in composition of antibody is no greater than 1 %, preferably no greater than 0.5 %. In a preferred aspect, the present invention provides a composition of pertuzumab wherein an amount of aggregates in composition of pertuzumab is no greater than 1 %, preferably no greater than 0.5 %. The aspects of the present invention also provides method of making composition of antibody, preferably pertuzumab, wherein the amount of aggregates in composition of pertuzumab is no greater than 1 %, preferably no greater than 0.5 %. No greater than 1 % includes no greater than 0.9 %, no greater than 0.8 %, no greater than 0.7 %, no greater than 0.6 %, no greater than 0.5 %, no greater than 0.4 %, no greater than 0.3 %, no greater than 0.2 %, no greater than 0.1 %.

In a furthermore aspect, a composition of antibody according to the present invention provides anti-proliferative activity, preferably, a composition of pertuzumab according to the present invention provides anti-proliferative activity. The anti-proliferative activity of pertuzumab composition was measured using $HER2^+/HER3^+$ expressing MCF-7 cells (human breast cancer cell line) in the presence of Heregulin (HRG) ligand. The inventors of the present invention has found that composition according to the present invention provides desired anti-proliferative activity of the antibody, preferably pertuzumab. The anti-proliferative activity of antibody was measured as % relative potency using *in-vitro* cell based anti-proliferation assay with fluorescence detection. The anti-proliferative activity of pertuzumab composition prepared according to the present invention is at least 99 % with respect to pertuzumab available in the market as measured by *in-vitro* cell based anti-proliferation assay with fluorescence detection. ADCC was also measured as % relative cytotoxicity for the composition prepared according to the present invention, preferably pertuzumab composition

by flow cytometry. The ADCC activity of pertuzumab composition prepared according to the present invention is at least 96 % with respect to pertuzumab available in the market as measured by flow cytometry.

In one of the aspects, present invention provides a method of making a composition of antibody wherein composition comprises antibody and reduced level of basic variants.

Preferably, present invention provides a method of making a composition of pertuzumab wherein composition comprises pertuzumab and reduced level of pertuzumab basic variants.

In one aspect, present invention provides a method of making a composition of pertuzumab wherein composition comprises pertuzumab and no more than 10 % of pertuzumab basic variants, preferably no more than 7 % of pertuzumab basic variants.

In further aspects, present invention provides a method of making a composition of antibody wherein composition has VHS- variant no more than 1 %, preferably no more than 0.5 %, more preferably no more than 0.2 %.

Preferably, present invention provides a method of making a composition of pertuzumab wherein composition has VHS- variant no more than 1 %, preferably no more than 0.5 %, more preferably no more than 0.2 %.

In one of the aspects, the method of making a composition of antibody with reduced level of basic variants is through a suitable purification process of the monoclonal antibody comprising cation exchange chromatography or multi-modal chromatography.

Preferably, the method of making a composition of pertuzumab with reduced level of pertuzumab basic variants according to the present invention is by using a suitable purification process of pertuzumab comprising cation exchange chromatography or multi-modal chromatography.

In one aspect, the method of making a composition of pertuzumab with pertuzumab basic variants no more than 10 %, preferably no more than 7 % according to the present invention is by using a suitable purification process of pertuzumab comprising cation exchange chromatography or multi-modal chromatography.

The present invention also provides a method of making a composition of antibody wherein antibody composition has antibody variant containing signal peptide residue(s) no more than 1 %, preferably no more than 0.5 %, more preferably no more than 0.2 %. An antibody variant containing signal peptide residue(s) according to the present invention is a VHS- variant.

Preferably, the method of making a composition of pertuzumab with pertuzumab variant containing signal peptide residue(s) no more than 1 %, preferably no more than 0.5 %, more preferably no more than 0.2 % is a purification process of monoclonal antibody comprising cation exchange chromatography or multi-modal chromatography.

In one aspect, the present invention provides a composition comprising antibody and reduced level of basic variants by cation exchange chromatography or multi-modal chromatography.

Preferably, the present invention provides a composition comprising pertuzumab and reduced level of pertuzumab basic variants by cation exchange chromatography or multi-modal chromatography.

In one aspect, the present invention provides a composition comprising pertuzumab and no more than 10 % of pertuzumab basic variants, preferably no more than 7 % of pertuzumab basic variants, wherein the said pertuzumab composition is achieved by cation exchange chromatography or multi-modal chromatography.

In one of the aspects, the present invention provides a composition comprising antibody and antibody variant containing signal peptide residue(s) wherein said variant is no greater than 1 % by cation exchange chromatography or multi-modal chromatography.

In one aspect, the composition of antibody with reduced level of basic variants is obtained by a process of purification, the method comprising following steps:

a) Affinity chromatography preferably, protein A chromatography
b) Cation exchange chromatography or multi-modal chromatography optionally, followed by other purification method such as chromatography method, UF/DF, microfiltration, and combination thereof.

Further chromatography method includes, but not limited to, anion exchange chromatography, cation exchange chromatography, HIC, mixed mode chromatography and the like. The said purification process provides antibody composition with no more than 10 of antibody basic variants.

More preferably, the composition of antibody with reduced level of basic variants is obtained when purified by a method comprising following steps:

a) Affinity chromatography preferably, protein A chromatography
b) Cation exchange chromatography or multi-modal optionally, followed by
c) Mixed mode chromatography

These chromatography steps can be a column chromatography or a membrane chromatography as is well known to a person skilled in the art. The said purification process provides antibody composition with no more than 10 % of antibody basic variants. Preferably, according to the present invention pertuzumab is prepared using purification process as described herein above.

In another aspect, the composition of antibody according to present invention is obtained when purified by a method comprising following steps:

a) Affinity chromatography preferably, protein A chromatography
b) Cation exchange chromatography or multi-modal chromatography optionally, followed by other purification method such as chromatography method, UF/DF, microfiltration, and combination thereof.

Further chromatography method includes, but not limited to, anion exchange chromatography, cation exchange chromatography, HIC, mixed mode chromatography and the like.

More preferably, the composition of antibody according to present invention is obtained through purification by a method comprising following steps:

a) Affinity chromatography preferably, protein A chromatography
b) Cation exchange chromatography or multi-modal optionally, followed by
c) Mixed mode chromatography

These chromatography steps can be a column chromatography or a membrane chromatography.

In further aspect, the composition of antibody according to present invention in obtained by the following purification steps:

a) Affinity chromatography preferably, protein A chromatography
b) Low-pH incubation
c) Neutralization and reconditioning by UF/DF
d) Cation exchange chromatography or multi-modal chromatography
e) Ultrafiltration-diafiltration
f) Mixed mode chromatography

Preferably, according to the present invention pertuzumab is prepared using purification process as described herein above.

Protein A is a protein originally found in the cell wall of the bacteria *Staphylococcus aureus*. Protein A chromatography step is useful to capture the monoclonal antibody from crude

mixture and to elute the desired monoclonal antibody from the column with high level of purity in bind-elute mode. Protein A chromatography as mentioned herein is performed as described in patent document WO2014/207763 published on 25 June 2014 along with suitable modifications as necessary which are within the scope of a person skilled in the art.

In one of the embodiments, the present invention provides a purification process of cell culture derived monoclonal antibody from a crude mixture by using a Protein A column chromatography, first to capture, and then elute the protein from the column with high level of purity at low pH optionally in the presence of additives/salts. Crude mixture may include host-cell derived contaminating proteins, DNA, product-related substances and other impurities in addition to that of the protein of interest. Protein G or protein L can be used as column matrix in the affinity chromatography step. Protein G is an immunoglobulin binding protein expressed in group C and G *Streptococcal* bacteria. Protein L is also an immunoglobulin binding protein isolated from surface of bacterial species *Peptostreptococcus magnus* which binds antibodies through light chain interactions. They are coupled with agarose base to form column matrix. For example, Protein G column comprises protein G covalently coupled with agarose base and protein L column comprises protein L which is covalently coupled with agarose base.

In another embodiment, the Protein A purification step according to the present invention includes three column wash steps wherein (i) first wash is with equilibration buffer, (ii) second wash at the same pH and/or at a conductivity higher than the first wash buffer (iii) Third wash at a pH and/or conductivity lower than the second wash (iv) Elution of the antibody at lower pH and/or higher conductivity than third wash.

In a preferred embodiment, the column wash steps according to the present invention comprises: (i) First wash with equilibration buffer at about pH 7.4 and/or conductivity about 20 mS/cm, preferably the conductivity is in the range of 1mS/cm to 30 mS/cm (ii) Second wash at about pH 7.4 and/or conductivity more than 20 mS / cm (iii) Third wash at a lower pH than pH 7.4, preferably in the range of pH 5 to pH 6.5 and/or conductivity less than 20 mS / cm, preferably in the range of 1 mS/cm to 10 mS / cm (iv) Elution of the antibody in the range of pH 3.0 – 4.5 and/or conductivity higher than 10 mS/cm.

In one of the embodiments, the buffer component for protein A chromatography purification step is selected from tris, phosphate, acetate and citrate buffers. Preparation and use of such

buffers and their compositions are known and are within the scope of a skilled person.

In a preferred embodiment, elution of the antibody according to the present invention is performed at a pH ranging from about pH 3.5 to pH 4, preferably pH 3.5 to pH 3.7.

In one of the embodiments, the process according to the present invention includes use of additives/salts selected from sodium chloride, arginine, glycine, preferably sodium chloride.

Cation exchange chromatography step or multi-modal chromatography is useful for removal of VHS- variant, other product-related variants and process-related impurities. It is performed preferably in bind-elute mode. A third chromatography step is optionally employed for further removal of product-related and process-related variants, if it is required. The third chromatography step can also be performed in flow-through-and-wash mode.

In another embodiment, the cation exchange chromatography step according to the present invention includes two column wash steps followed by elution step wherein (i) First wash is carried out with equilibration buffer (ii) Second wash is carried out at the same pH and/or at a conductivity higher than the first wash buffer (iii) Elution of the antibody is carried out at same pH and/or higher conductivity than the second wash. Eluted protein is collected in two fractions as described in Example 2 provided herein below.

The present invention provides removal of residual process-related and product-related variants from the desired protein fraction by using an ion exchange column chromatography, preferably cation exchange chromatography in bind-elute mode. Elution of the desired protein is performed with increasing salt concentration. Ion exchange chromatography, preferably cation exchange chromatography can also be performed in flow-through-and-wash mode. The column matrix for cation exchange chromatography according to the present invention is selected from SP Sepharose, SP-5PW, Capto™ SP impRes and PARESTO SP35 Jetted. Preferably, column matrix for cation exchange chromatography according to the present invention is Capto™ SP impRes.

SP Sepharose is an agarose based cation exchanger with a sulfopropyl group.

Capto™ SP impRes is a cation exchanger for high-throughput intermediate purification and polishing steps of biomolecules. The chromatography media is based on the high-flow agarose Capto product line provides good pressure/flow properties and a small bead size (approx. 40μm) that gives high resolution. The ligand is a well-established SP ligand; a sulfopropyl group.

SP-5PW is a cation exchange resin for biomolecule purification. It is composed of highly

crosslinked polymethacrylate beads that have been functionalized with sulfopropyl (SP) strong cation exchange groups.

PARESTO SP35 Jetted is an agarose-based, acid cation exchange resin designed for biomolecule purification, including proteins, peptides and oligonucleotides. It is manufactured using 'Jetting' method that produces agarose beads with a very narrow particle size distribution.

The salt for elution of the desired protein at ion exchange chromatography step according to the present invention is selected from sodium chloride, ammonium chloride and sodium sulphate preferably, sodium chloride. Ion exchange chromatography is performed at pH in the range of pH 3 to pH 8. For example, ion exchange chromatography is performed about at pH 3, at pH 4, at pH 5, at pH 6, at pH 7 or pH 8.

Multi-modal chromatography according to the present invention is selected from strong cation exchange-weak cation exchange-strong anion exchange multimodal chromatography. The present invention provides further removal of residual process-related and product-related variants from the desired protein fraction by using multi-modal chromatography in bind-elute mode or flow-through-and-wash mode. The column matrix for Multi-modal chromatography step is BAKERBOND^{TM} XWP 500 Poly CSX. PolyCSX as described herein is a multi-mode ion exchanger which primarily functions as a strong cation exchanger over a wide pH range. Its strong cationic exchange sites are a result of sulfonic acid groups. Weak cationic exchange sites are due to carboxylic acid groups, while weak anion exchange behavior is maintained by the presence of amino groups on the Polyethylenimine (PEI) ligands. PolyCSX contains -NH-C (=O)-CH2-CH (SO_3H) (CO_2H) and equivalent variations as a functional group.

Mixed mode chromatography is performed herein for removal of product-related and process related variants. It can be performed in bind-elute or flow-through-and-wash mode. Preferably, the mixed mode column comprising hydrophobic interaction resin and anion exchange resin are used in the present invention. In another embodiment, column matrix for mixed mode chromatography step is selected from Capto adhere impress, Diamond MIX–A Mustang, Poly Hi-Propyl, etc. Capto adhere impRes has been used in the present invention. The column matrices referred herein this application are as follows.

Capto adhere impress is a mixed mode chromatography resin for purification of monoclonal antibodies and other recombinant/native proteins by packed bed chromatography. In Capto

adhere impRes, ligand N-benzyl N-methyl ethanolamine is coupled to an activated base matrix to form the mixed mode anion exchanger.

Diamond MIX–A Mustang is a multi-mode strong anion exchange medium formed by coupling phenyl glycidyl ether and 2, 3-epoxypropyl trimethylammonium chloride on small and highly rigid

agarose microspheres through two crosslinks.

Poly HI-Propyl media is a mixed-mode Hydrophobic Interaction Chromatography (HIC) media with unique selectivity resulting from a proprietary surface chemistry having both hydrophobic and weak anionic exchange sites.

Preferably, purification of the desired monoclonal antibody derived from cell culture according to the present invention is sequentially carried out as follows:

a) Affinity chromatography preferably, protein A chromatography
b) Cation exchange chromatography or multi-modal chromatography
c) Mixed mode chromatography

More preferably, purification of the desired monoclonal antibody derived from cell culture is performed sequentially as follows –

a) Affinity chromatography preferably, protein A chromatography
b) Low-pH incubation
c) Neutralization and reconditioning by ultrafiltration-diafiltration
d) Cation exchange chromatography or multi-modal chromatography
e) Ultrafiltration-diafiltration
f) Mixed mode chromatography

Diafiltration medium according to the present invention is selected from water, tris, citrate buffer, phosphate buffer, succinate buffer, acetate buffer and combination thereof.

Recovery of antibody from the cation exchange column matrix, according to the present invention is performed with additive/salt selected from sodium chloride, arginine, glycine, L-Histidine, preferably sodium chloride.

The process of the present invention can be suitably modified by a skilled person to prepare other antibody composition with reduced level of antibody variants as described elsewhere in the specification. Such antibody variants of antibodies described in the current application, as can be prepared by the process of the present invention are meant to be encompassed within the scope of

the present invention.

The composition prepared according to the present invention may further include acidic variants of the antibody. Such acidic variants elute before the principal peak of the main antibody as assessed by HP-IEC. Also, the composition prepared according to the present invention can be formulated with pharmaceutically acceptable excipients or carriers to prepare a pharmaceutical formulation that can be used for therapeutic purpose.

The process for preparing the antibody compositions according to the present invention are further described in detail below:

I) Protein A column chromatography:

Protein A is carried out as per the general process described in patent document WO2014/207763 published on 25 June, 2014. Cell culture derived clarified supernatant containing the desired monoclonal antibody and other contaminants is loaded on to a Protein A column equilibrated with a suitable buffer at a pH close to neutrality. The desired monoclonal antibody binds to the affinity matrix, whereas majority of the contaminants pass out of the column in the flow-through. Prior to the elution of the desired protein, the column is washed with a plurality of wash steps. The first wash is performed after the completion of column-loading with the same equilibration buffer. The second wash is performed with a buffer of the same pH having higher conductivity than that of the first wash buffer. The third wash is carried out at a different pH and conductivity buffer than that of the first and second wash steps. Elution of the desired protein is carried out at pH lower than that of the third wash step, but at higher conductance. Finally, column cleaning is performed with an alkaline solution.

II) Cation exchange column chromatography:

Strong cation exchange is used for further purification of antibody in bind-elute mode. This chromatography step is performed mainly to remove the product related variant, specifically VHS- variant from antibody composition along with some process-related impurities. Upon binding to the column matrix, antibody is eluted with a salt-gradient in a step-wise manner. Majority of VHS- variant is observed to remain bound tightly to the matrix and passes out of the column after elution of the desired protein (main peak). Elution of the desired protein is carried out in the presence of 100 mM NaCl and majority of the VHS- variant is washed out of the column in the subsequent wash step with 300

mM NaCl. The column eluted peak is collected in two fractions – the major fraction Fr 1 containing the desired level of purity is used for further processing. Majority of the VHS- variant is washed out of the column after protein elution as a separate peak in the presence of 300 mM NaCl. Eluted fraction, Fr 1 was analyzed by HP-SEC and HP-IEC to assess the level of purity (> 99.5 %) and charge heterogeneity (including VHS- variant; < 0.2 %) profiles to ensure the product quality. The amount of aggregates in eluted fraction of antibody composition, preferably pertuzumab composition, is no greater than 1 %, preferably, no greater than 0.5 %.

Multi-modal column chromatography:

A multi-modal column matrix, comprising strong cation, weak cation and strong anion exchange (BAKERBOND™ XWP 500 Poly CSX,) functional groups was used for further purification of pertuzumab protein in bind-elute mode. This chromatography step is performed to remove the residual product- and process-related impurities, like the basic variants including the VHS- variant from the antibody preparation and HCP. Upon binding to the column matrix, antibody is eluted with increasing concentration of salt-gradient in a step-wise manner. Antibody preparation with desired quality is found to elute out of the column with 50 mM NaCl in 20 mM Na-citrate buffer of pH 5.0, while majority of VHS- variant impurity is observed to remain bound tightly to the matrix and is washed out with higher salt concentration (300 mM NaCl). The desired antibody recovered with the eluted above specified eluted fraction was analyzed by HP-SEC and HP-IEC to assess the level of purity (> 99.5 %) and charge heterogeneity (VHS- variant; < 0.2 %) profiles to ensure the product quality. The amount of aggregates in eluted fraction of antibody composition, preferably pertuzumab composition, is no greater than 1 %, preferably, no greater than 0.5 %.

III) Mixed mode column chromatography:

After the second column step as mentioned above, solution containing the desired monoclonal antibody is reconditioned substantially to match up to the pH and conductivity of the mixed mode column equilibration conditions. Column is equilibrated with a buffer of pH about 7.4. The desired protein is recovered from the column in the flow-through-and-wash fraction. For carrying out mixed mode chromatography according to the present invention, other mixed mode which also can be used are selected from Capto adhere

impRes, Diamond MIX –A Mustang, Capto MMC, Poly Hi-Propyl, Toyopearl MX-Trp-650M, Eshmuno HCX media, etc. Capto Adhere ImpRes has been used in the present invention.

Analytical methods used in the present invention:

HP-Size exclusion chromatography (HP-SEC):

Samples were analyzed to estimate the high molecular weight species or aggregates and low molecular weight or fragments by HP-size exclusion chromatography (HP-SEC) using TSK gel G3000 SWXL column (7.8 mm I.D × 30 cm L). Samples were loaded and eluted isocratically using sodium phosphate buffer at a flow rate of 0.5 mL / min. Elution was monitored at UV 215 nm.

HP- Ion Exchange Chromatography (HP-IEC):

Separation of different charge variants of the purified monoclonal antibody is carried out by HP-IEC. The column is equilibrated in sodium phosphate buffer of pH 6.9 (mobile phase A). Elution of the charge variants of the said protein is carried out with increasing salt concentration (sodium chloride) in mobile phase A at 0.5 mL/min.

The present invention is illustrated with the following non-limiting examples which should not be interpreted as limiting the scope of the invention in any way:

Example 1: Preparation of pertuzumab composition free from VHS- variant using Multi mode chromatography

Step 1: Cell separation / clarification / reconditioning

After harvesting the batch, cells were separated from the culture broth by centrifugation followed by depth filtration in order to obtain clear supernatant containing the protein of interest along with other soluble contaminants. Centrifugation was carried out at 10,000 g × 30 minutes. Depth filtration was performed by using a 0.45 + 0.2 µm (combined) membrane. The cell-free clarified supernatant was recovered and reconditioned to tune up with the Protein A column equilibration buffer conditions for pH and conductivity.

Step 2: Capture step by r-PA column chromatography

After reconditioning, the clarified supernatant containing the desired protein was passed through the Protein A column to capture the pertuzumab (IgG1) monoclonal antibody by the affinity matrix. Prior to loading, the column was equilibrated with a suitable buffer of pH

about 7.4 and conductivity about 20 mS /cm. Subsequent to loading, the column was washed with the same buffer (first wash). Following the equilibration buffer wash step, the column was washed with the same buffer of pH 7.4 but at higher conductivity (second wash); > 25 mS/cm. A third wash step was performed with a second buffer of pH about 5.5 but at a third conductivity in the range of 5 – 10 mS / cm. Following the third column wash step, elution of the desired protein, pertuzumab was conducted with a suitable buffer at a third pH of about 3.5. Pertuzumab eluted at this step was observed to exhibit > 98 % purity as assessed by analytical HP-SEC.

Step 3: Low-pH incubation

r-PA column-eluted desired protein fraction was incubated at low pH condition, pH 3.0 – 4.0 for at least 45 min under room temperature conditions for potential viral inactivation, after which the protein solution was passed through a 0.2 µm filter.

Step 4: Neutralization and reconditioning

Following low-pH treatment, pH-neutralization of the protein solution was performed with the addition of an alkaline solution, like tris base or Na-citrate or ammonium hydroxide, in a controlled manner, and adjusted to pH about 5.0 – 6.8.

Step 5: Reconditioning by UF / DF

The pH-neutralized protein solution was reconditioned further by ultrafiltration-diafiltration (8 – 10 dia-volumes) with the adjustment of pH and conductivity using 30 kDa MWCO membrane filter to match up to the next column equilibration conditions. After reconditioning, protein solution was passed through a 0.2 µm membrane filter and loaded on to a multi-modal column.

Step 6: Multi-modal column chromatography:

A second column, multi-modal in nature, comprising strong cation, weak cation and strong anion exchangers was used for further purification of pertuzumab protein in bind-elute mode. This column step was performed mainly to remove the product related impurities, like the basic variants including the VHS- variant from the preparation along with some process-related impurities. Upon binding to the column matrix, pertuzumab protein was eluted with a salt-gradient in a step-wise manner. Pertuzumab protein with desired quality was found to elute out of the column with 50 mM NaCl in 20 mM Na-citrate buffer of pH 5.0, while

majority of VHS- variant impurity was observed to remain bound tightly to the matrix and was washed out of the column separately with higher salt concentration (300 mM NaCl). Column eluted major fraction was analyzed by HP-SEC and HP-IEC to assess the level of purity and charge heterogeneity, respectively. Results of HP-IEC analysis is provided in table 1. No greater than 0.5 % aggregates of pertuzumab (more than 99.5 % purity of pertuzumab) in pertuzumab composition was achieved with the column eluted major fraction after this column step, as assessed by HP-SEC. VHS- variant was no more than 0.5 % in purified pertuzumab composition after this column step as shown in Figure 1. The same results was also observed for the CpB treated purified pertuzumab composition as shown in Figure 3.

Basic variants of pertuzumab was observed to be < 5 % in the composition prepared in example 1 which is significantly lesser than the basic variants of pertuzumab identified in the reference product (> 13 %).

Table 1: charge – variant profile of pertuzumab composition as analysed by HP-IEC

Sample Name	Acidic variants (%)	Principal peak (%)	Basic variants (%)	VHS- variants (%)
Multi-mode load	25.53	65.39	5.03	4.05
Multi-mode elution	30.69	65.46	3.87	Not detected

Example 2: Preparation of pertuzumab composition free from VHS- variant using cation exchange chromatography

Step 1: Cell separation / clarification / reconditioning

After harvesting the batch, cells were separated from the culture broth by centrifugation followed by depth filtration in order to obtain clear supernatant containing the protein of interest along with other soluble contaminants. Centrifugation was carried out at 10,000 g × 30 minutes. Depth filtration was performed by using a 0.45 + 0.2 µm (combined) membrane. The cell-free clarified supernatant was reconditioned to tune up with the Protein A column equilibration buffer conditions for pH and conductivity.

Step 2: Capture step by Protein A column chromatography

After reconditioning, the clarified supernatant containing the desired protein was passed through the Protein A column to capture the pertuzumab (IgG1) monoclonal antibody by the affinity matrix. Prior to loading, the column was equilibrated with a suitable buffer of pH about 7.4 and conductivity about 20 mS /cm. Subsequent to loading, the column was washed with the same buffer (first wash). Following the equilibration buffer wash step, the column was washed with the same buffer of pH 7.4 but at higher conductivity (second wash); > 25 mS / cm. A third wash step was performed with a second buffer of pH 5.5 at a third conductivity in the range of 5 – 10 mS / cm. Following the third column wash step, elution of the desired protein, pertuzumab was conducted with a suitable buffer of a third pH value in the range of about 3.5. Pertuzumab eluted at this step was observed to exhibit at least 98 % purity as assessed by analytical HP-SEC.

Step 3: Low-pH incubation

r-PA column-eluted desired protein fraction was incubated at low pH condition, pH 3.0 – 4.0 for at least 45 min under room temperature conditions for viral inactivation, after which the protein solution was passed through a 0.2 μm filter.

Step 4: Neutralization and reconditioning

Following low-pH treatment, pH-neutralization of the protein solution was performed with the addition of an alkaline solution tris base, in a controlled manner, and pH was adjusted to the desired level of about pH 5.0 – 6.8. Na citrate or ammonium hydroxide can also be used as an alkaline solution.

Step 5: Reconditioning by UF / DF

The pH-neutralized protein solution was reconditioned further by ultrafiltration-diafiltration (8 – 10 dia-volumes) with the adjustment of pH and conductivity using 30 kDa MWCO membrane filter to match up to the next column equilibration conditions. After reconditioning, protein solution was passed through a 0.2 μm membrane filter and loaded on to a SCEX column.

Step 6: Cation exchange column chromatography:

A second column, comprising strong cation exchange matrix was used for further purification of pertuzumab protein in bind-elute mode. This column step was performed mainly to remove

the product related impurities, like the basic variants including the VHS- variant from the preparation along with some process-related impurities. Upon binding to the column matrix, pertuzumab protein was eluted with a salt-gradient in a step-wise manner. Pertuzumab protein with desired quality was found to elute out of the column with 100 mM NaCl in 20 mM Na-citrate buffer of pH 5.0. The column eluted peak is collected in two fractions and the major fraction, Fr 1 containing the desired level of purity is used for further processing. Majority of other VHS- basic variants was observed to remain bound tightly to the matrix and was washed out of the column subsequently in high salt concentration (300 mM NaCl) with a separate peak.

Fr 1 was analyzed by HP-SEC and HP-IEC to assess the level of purity and charge heterogeneity (VHS- variant) profiles to ensure the product quality. Results of HP-IEC analysis is provided in table 2. No greater than 0.5 % aggregates of pertuzumab (more than 99.5 % purity of pertuzumab) was achieved after this column step, as assessed by HP-SEC. VHS- variant of pertuzumab was no more than 0.5 % after this column step, as assessed by HP-IEC as shown in Figure 2. The same results was also observed for the CpB treated purified pertuzumab composition as shown in Figure 4.

Table 2: charge – variant profile of pertuzumab composition as analysed by HP-IEC

Sample Name	Acidic Variants (%)	Principal peak (%)	Basic variants (%)	VHS- Variants (%)
SCEX Load	26.11	65.62	5.48	2.79
SCEX FR 1	26.36	71.58	2.05	Not detected

Step 7: Ultrafiltration-diafiltration (UF/DF)

The cation exchange column-eluted fraction was reconditioned, substantially, by UF / DF using 30 kDa MWCO membrane filter against low ionic strength tris buffer solution of pH 7.4 in order to match to the equilibration buffer conditions (e.g. pH and conductivity) of the next column (mixed mode column) step. Diafiltered protein solution was passed through a 0.2 µm filter and loaded on to a mixed mode column.

Step 8: Mixed mode column chromatography

Diafiltered protein solution containing the desired monoclonal antibody was passed through a mixed mode column equilibrated with 25 mM Tris-Cl; pH 7.4; conductivity 14 mS / cm. The desired protein was recovered in flow-through-and-wash fraction. After the mixed mode purification step, purity of pertuzumab was observed to be > 99.5 %, as assessed by HP-SEC. The purified preparation may then be suitably formulated for use as a pharmaceutical substance for human use. As illustrated above in examples, present invention provides pertuzumab composition wherein VHS- variant of pertuzumab was no more than 0.5 %.

Basic variants of pertuzumab was observed to be < 5 % in the composition prepared in example 2 which is significantly lesser than the basic variants of pertuzumab identified in the reference product (> 13 %).

Incorporation by reference

The entire disclosure of each of the patent documents and scientific articles referred to herein is incorporated by reference for all purposes.

Equivalents

The invention may be embodied in other specific forms without departing from the spirit or essential characteristics thereof. The foregoing embodiments are therefore to be considered in all respects illustrative rather than limiting the invention described herein. Scope of the invention is thus indicated by the appended claims rather than by the foregoing description, and all changes that come within the meaning and range of equivalency of the claims are intended to be embraced therein.

We claim:

1. A composition comprising pertuzumab and pertuzumab basic variants wherein basic variants is no more than 10 %.
2. The composition as claimed in claim 1, wherein the basic variants is no more than 7 %.
3. The composition as claimed in claim 1, wherein the basic variants in the composition includes pertuzumab variant containing signal peptide residue(s).
4. The composition as claimed in claim 3, wherein the pertuzumab variant containing signal peptide residue(s) in the composition is no more than 1 %.
5. The composition as claimed in claim 4, wherein the pertuzumab variant containing signal peptide residue(s) in the composition is no more than 0.5 %, preferably no more than 0.2 %.
6. The composition as claimed in claim 4, wherein composition is free from pertuzumab variant containing signal peptide residue(s).
7. The composition as claimed in claim 3, wherein the pertuzumab variant containing signal peptide residue(s) in the composition is a VHS- variant.
8. The composition as claimed in claim 7, wherein the VHS- variant is no more than 1 %,
9. The composition as claimed in claim 8, wherein the VHS- variant is no more than 0.5 %, preferably no more than 0.2 %.
10. The composition as claimed in claim 7, wherein the composition is free from VHS- variant.
11. A method of making composition of pertuzumab with no more than 10 % pertuzumab basic variants wherein the said method comprises chromatography.
12. The method as claimed in claim 11, wherein pertuzumab basic variants in the composition includes pertuzumab variant containing signal peptide residue(s).
13. The method as claimed in claim 11, wherein the chromatography is selected from affinity chromatography, cation exchange chromatography, mixed mode chromatography, multi-mode chromatography and/or combination thereof.
14. The method as claimed in claim 13 comprises following steps:
    a) Affinity chromatography preferably, protein A chromatography
    b) Cation exchange chromatography or multi-modal chromatography optionally followed by,

c) Mixed mode chromatography

15. The method as claimed in claim 14 comprises following steps:

a) Affinity chromatography preferably, protein A chromatography

b) Low-pH incubation

c) Neutralization and reconditioning by ultrafiltration-diafiltration

d) Cation exchange chromatography or multi-modal chromatography

e) Ultrafiltration-diafiltration

f) Mixed mode chromatography

16. The method as claimed in claim 11 to claim 15 wherein the pertuzumab variant containing signal peptide residue(s) is VHS- variant.

17. The composition of pertuzumab as claimed in claim 1 to claim 10 wherein an amount of aggregates in composition of pertuzumab is no greater than 1 %, preferably no greater than 0.5 %.

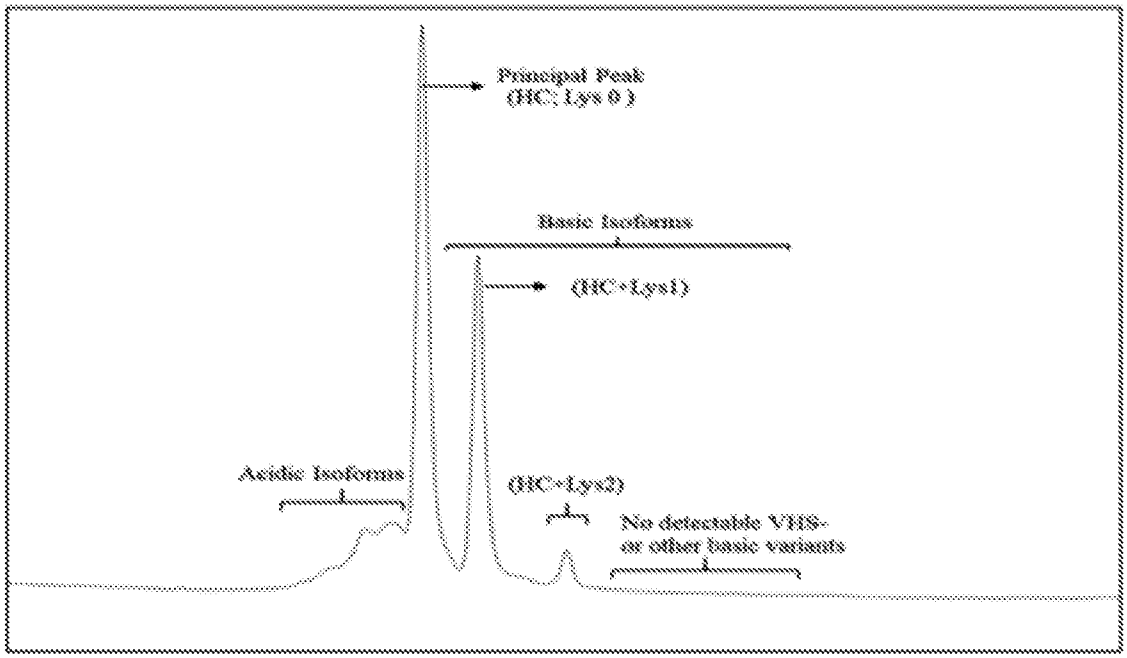


Figure: 1

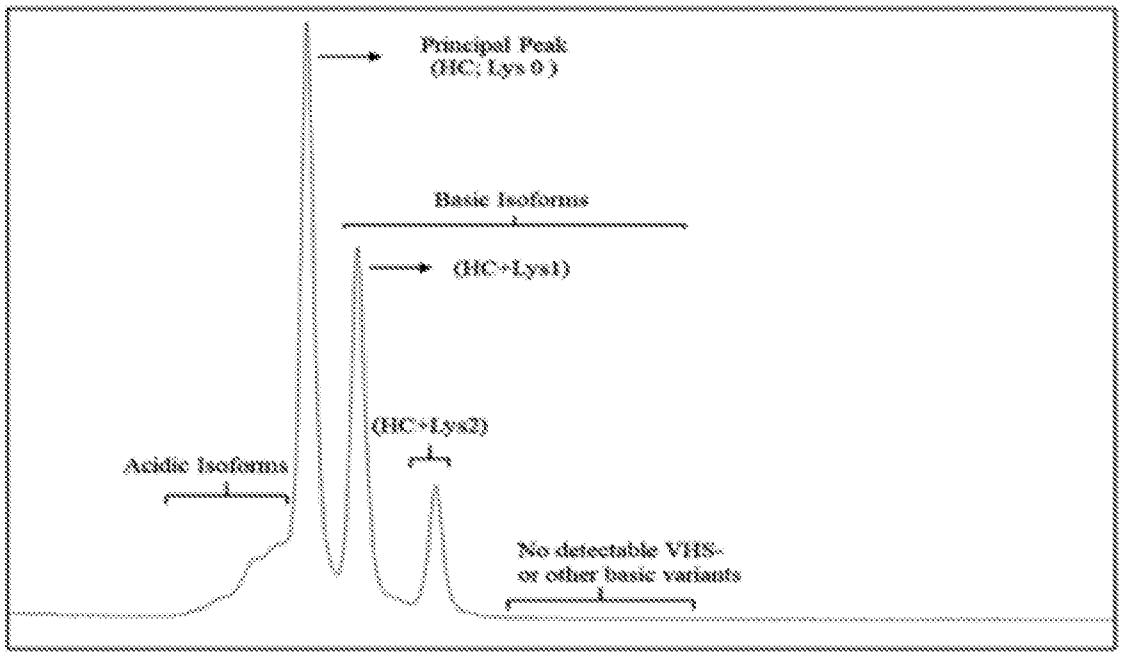


Figure: 2

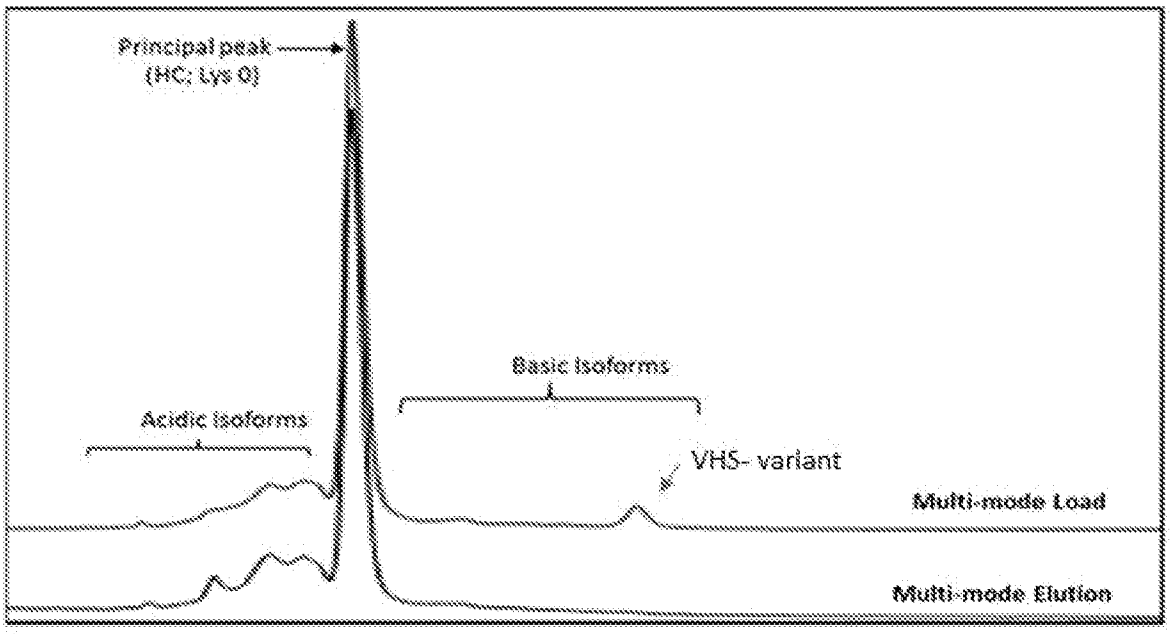


Figure: 3

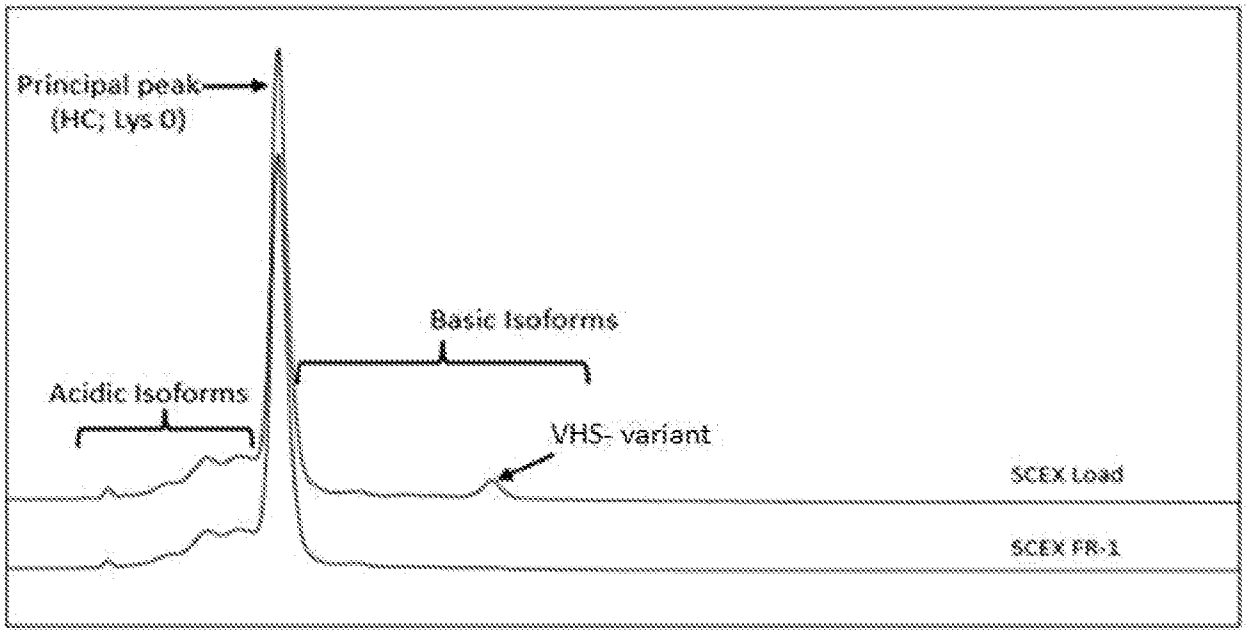


Figure: 4

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2019/059043

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/32 A61K39/395 A61P35/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2009/099829 A1 (GENENTECH INC [US]; HARRIS REED J [US]; MOTCHNIK PAUL A [US]) 13 August 2009 (2009-08-13) page 1, line 16 - line 19 page 5, line 9 - line 15 page 6, line 12 - line 34 page 7, line 13 - line 27; figures 2-5 page 8, line 10 - line 11; figure 12 example 1, in particular page 67, line 1-page 68, line 24; page 33, line 17 - page 35, line 15; claims 1-9,16-27	1-17
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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 January 2020	28/01/2020

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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1

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