DESCRIPTION

TITLE OF INVENTION

PHARMACEUTICAL COMPOSITION COMPRISING ANTI-HUMAN TSLP RECEPTOR ANTIBODY

TECHNICAL FIELD

[0001]

The present invention relates to a stable pharmaceutical composition comprising an anti-human TSLP receptor antibody. Further, the present invention relates to a stable and highly concentrated pharmaceutical composition comprising an anti-human TSLP receptor antibody.

BACKGROUND ART

[0002]

It is known that a monoclonal antibody that specifically binds to a human TSLP (thymic stromal lymphopoietin) receptor and inhibits the action of human TSLP via the human TSLP receptor is useful for prevention and/or treatment of various diseases in which human TSLP and a human TSLP receptor are involved in disease pathology (for example, prevention and/or treatment of asthma)(Patent literature 1).

[0003]

Patent literature 1 discloses fully human type T7-27 as an anti-human TSLP receptor antibody, and discloses that it inhibits the expression of TARC (thymus and activation-regulated chemokine) mRNA induced by TSLP and the production of MDC (macrophage-derived chemokine) protein, and that it suppresses an allergic reaction in a monkey ascaris antigen sensitization model.

[0004]

On the other hand, various antibody medicines have been developed in recent years, and are actually provided for the medical field. Many antibody medicines are administered by intravenous administration, subcutaneous administration, or the like, and thus are provided as a form of a parenteral pharmaceutical composition, such as a liquid preparation, a lyophilized preparation, or the like, for the medical field. Since parenteral pharmaceutical compositions are presumed to be directly administered into the body, stable pharmaceutical preparations are required.

[0005]

Also, from a pharmaceutical viewpoint, since intravenous administration or subcutaneous administration is assumed as the route of administration, in the case of making the same pharmaceutical preparation, taking into consideration the dose for

subcutaneous administration, it is desirable that the pharmaceutical preparation is a high concentration preparation.

[0006]

However, in a solution containing a high concentration of antibody, undesirable phenomena may occur, including the formation of insoluble aggregates and/or soluble aggregates. The insoluble aggregates and soluble aggregates are formed in a solution state by the association of antibody molecules. Further, when the solution is stored for a long period of time, deamidation of an asparagine residue may decrease the physiological activity of the antibody molecule.

[0007]

As a technique concerning a highly concentrated antibody and a protein preparation, an invention relating to a stable, liquid preparation of low turbidity comprising a protein or antibody in an amount of 100 to 260 mg/mL, arginine hydrochloride in an amount of 50 to 200 mmol/L, histidine in an amount of 10 to 100 mmol/L, polysorbate in an amount of 0.01 to 0. 1%, wherein the preparation has a pH ranging from 5.5 to 7.0, a kinematic viscosity of about 50 cs or less, and osmolarity ranging from 200 mOsm/kg to 450 mOsm/kg, is known (Patent literature 2). However, the anti-human TSLP receptor antibody is not disclosed nor suggested in Patent literature 2.

[0008]

Patent literature 3 discloses an invention relating to a stable pharmaceutical composition containing an anti-TSLP antibody, but it is not an invention relating to an anti-TSLP receptor antibody.

CITATION LIST

PATENT LITERATURE

[0009]

[Patent literature 1] WO 2015/020193
[Patent literature 2] WO 2004/091658
[Patent literature 3] WO 2014/031718

SUMMARY OF INVENTION

TECHNICAL PROBLEM

[0010]

An object of the present invention is to provide a stable pharmaceutical composition comprising fully human type T7-27, which is an anti-human TSLP receptor antibody.

More particularly, an object of the present invention is to provide a pharmaceutical composition comprising fully human type T7-27, which is an anti-human

TSLP receptor antibody, for example, (i) a pharmaceutical composition capable of suppressing the generation of chemically modified substances, such as deamidated forms and oxidized forms, or degradants or multimers, which increase due to heat, (ii) a pharmaceutical composition capable of suppressing the generation of oxidized forms, promoted by the addition of arginine, (iii) a pharmaceutical composition capable of suppressing the generation of microparticles, which increase after physical stress, or (iv) a pharmaceutical composition capable of suppressing the generation of oxidized forms, which increase when the concentration of a surfactant is high.

SOLUTION TO PROBLEM

[0011]

The present inventors found that a stable pharmaceutical composition could be prepared by formulating fully human type T7-27, which was an anti-human TSLP receptor antibody, in an arginine solution (Example 2 below), that a stable pharmaceutical composition could be prepared by adjusting the pH of the solution to an appropriate range, or using various buffer components (Examples 1, 3, and 4 below), and a further stable pharmaceutical composition could be prepared by using a surfactant, adjusting the antibody concentration, or the like, and completed the present invention.

[0012]

The present invention relates to:

[1] a stable pharmaceutical composition comprising an anti-human TSLP receptor antibody, a pharmaceutically acceptable buffer, arginine or a pharmaceutically acceptable salt thereof, and a surfactant, wherein the following (1) and/or (2) is contained as the anti-human TSLP receptor antibody:

(1) an anti-human TSLP receptor antibody comprising a heavy chain consisting of the amino acid sequence of SEQ ID NO: 1, and a light chain consisting of the amino acid sequence of SEQ ID NO: 3, and

(2) an anti-human TSLP receptor antibody consisting of amino acid sequences of an antibody generated by a post-translational modification of the anti-human TSLP receptor antibody of (1);

[2] the pharmaceutical composition of [1], wherein the pharmaceutically acceptable buffer is one, or two or more selected from the group consisting of phosphoric acid, citric acid, acetic acid, succinic acid, histidine, ascorbic acid, glutamic acid, lactic acid, maleic acid, trometamol, and gluconic acid;

[3] the pharmaceutical composition of [1] or [2], wherein the pharmaceutically acceptable buffer is phosphoric acid;

[4] the pharmaceutical composition of any one of [1] to [3], wherein the concentration of the pharmaceutically acceptable buffer is 5 to 100 mmol/L;

[5] the pharmaceutical composition of any one of [1] to [4], wherein the pharmaceutical

composition is a liquid preparation, or a lyophilized preparation or a spray-dried preparation;
[6] the pharmaceutical composition of [5], wherein the pharmaceutical composition is a liquid preparation;
[7] the pharmaceutical composition of [5], wherein when the pharmaceutical composition is a liquid preparation, a pH of the liquid preparation is 5 to 6, or when the pharmaceutical composition is a lyophilized preparation or a spray-dried preparation, a pH of a solution after reconstituting the preparation in water is 5 to 6;
[8] the pharmaceutical composition of [7], wherein the pharmaceutical composition is a liquid preparation, and the pH of the liquid preparation is 5 to 6;
[9] the pharmaceutical composition of any one of [1] to [8], wherein the concentration of the arginine or a pharmaceutically acceptable salt thereof is 700 mmol/L or less;
[10] the pharmaceutical composition of any one of [1] to [9], wherein the surfactant is one, or two or more selected from the group consisting of polysorbates and poloxamer 188;
[11] the pharmaceutical composition of any one of [1] to [10], wherein the surfactant is polysorbate 80;
[12] the pharmaceutical composition of any one of [1] to [11], wherein a content of the surfactant is 0.001 to 1% (w/v);
[13] the pharmaceutical composition of any one of [1] to [12], wherein the content of the surfactant is 0.01 to 0.2% (w/v);
[14] the pharmaceutical composition of any one of [5] to [13], wherein when the pharmaceutical composition is a liquid preparation, a content of the anti-human TSLP receptor antibody is 0.007 to 2 mmol/L, or when the pharmaceutical composition is a lyophilized preparation or a spray-dried preparation, a content of a solution after reconstituting the preparation in water is 0.007 to 2 mmol/L;
[15] the pharmaceutical composition of any one of [1] to [14], when the pharmaceutical composition is stored, each content of a degradant and a multimer is 10% or less, or a content of a chemically modified substance is 50% or less;
[16] the pharmaceutical composition of [1], comprising an anti-human TSLP receptor antibody comprising a heavy chain consisting of the amino acid sequence of SEQ ID NO: 1, and a light chain consisting of the amino acid sequence of SEQ ID NO: 3;
[17] the pharmaceutical composition of [1], comprising an anti-human TSLP receptor antibody consisting of amino acid sequences of an antibody generated by a post-translational modification of an anti-human TSLP receptor antibody comprising a heavy chain consisting of the amino acid sequence of SEQ ID NO: 1, and a light chain consisting of the amino acid sequence of SEQ ID NO: 3;
[18] the pharmaceutical composition of [1], comprising an anti-human TSLP receptor antibody comprising a heavy chain consisting of the amino acid sequence of amino acids 1-447 of SEQ ID NO: 1, and a light chain consisting of the amino acid sequence of SEQ

ID NO: 3; and
[19] the pharmaceutical composition of [1], comprising an anti-human TSLP receptor antibody comprising a heavy chain consisting of the amino acid sequence of SEQ ID NO: 1, and a light chain consisting of the amino acid sequence of SEQ ID NO: 3, and an anti-human TSLP receptor antibody comprising a heavy chain consisting of the amino acid sequence of amino acids 1-447 of SEQ ID NO: 1, and a light chain consisting of the amino acid sequence of SEQ ID NO: 3.

ADVANTAGEOUS EFFECTS OF INVENTION

[0013]

According to the present invention, a stable pharmaceutical composition comprising fully human type T7-27, which is an anti-human TSLP receptor antibody, more particularly, a stable pharmaceutical composition comprising an anti-human TSLP receptor antibody, capable of suppressing the generation of chemically modified substances, such as deamidated forms and oxidized forms, or degradants or multimers, or the generation of microparticles, can be provided.

BRIEF DESCRIPTION OF THE DRAWINGS

[0014]

[Fig. 1-1] Fig. 1-1 shows the evaluation results of SEC and IEC obtained in Example 1 (SEC multimers).
[Fig. 1-2] Fig. 1-2 shows the evaluation results of SEC and IEC obtained in Example 1 (SEC degradants).
[Fig. 1-3] Fig. 1-3 shows the evaluation results of SEC and IEC obtained in Example 1 (IEC main peak).
[Fig. 2] Fig. 2 shows the evaluation results of SEC obtained in Example 2.
[Fig. 3-1] Fig. 3-1 shows the results of arginine and pH in the group of phosphoric acid-containing formulations obtained in Example 3.
[Fig. 3-2] Fig. 3-2 shows the results of arginine and pH in the group of histidine-containing formulations obtained in Example 3.
[Fig. 4-1] Fig. 4-1 shows the evaluation results of HIC obtained in Example 5.
[Fig. 4-2] Fig. 4-2 shows the evaluation results of HIC obtained in Example 5.
[Fig. 5] Fig. 5 shows the evaluation results of viscosity obtained in Example 6.

DESCRIPTION OF EMBODIMENTS

[0015]

The term “stable” as used herein means to have stability against, for example, heat, light, temperature, and/or humidity. For example, after a pharmaceutical composition is allowed to stand under predetermined conditions, it means that impurities

contained in the pharmaceutical composition, for example, chemically modified substances, such as deamidated forms and oxidized forms, or degradants or multimers, are a specific amount or less. The amounts are defined as percentage (%), by measuring the area of multimer peaks or degradant peaks detected by SEC by an automatic analysis method and dividing it by the sum of all peak areas including the main peak, by measuring the area of the main peak detected by IEC by an automatic analysis method and dividing it by the sum of all peak areas other than the main peak, or by measuring the area of a hydrophilic peak detected by HIC by an automatic integration method and dividing it by the sum of all peak areas including the main peak. The main peak as used herein means the peak of the active body.

[0016]

The chemically modified substance is a structure generated by a chemical modification of part of the sequence of the antibody molecule. The amount of the chemically modified substances is defined as 0% to 70% as one embodiment, and 0% to 50% as another embodiment.

[0017]

The deamidated form is a chemically modified substance in which a portion of the amino residues of the antibody molecule undergo a deamidation reaction. A measuring method of the deamidated form is not particularly limited, so long as the deamidated form can be measured. The measuring method includes, for example, ion exchange chromatography and the like. The amount of the deamidated forms is defined as 0% to 70% as one embodiment, 0% to 50% as another embodiment, and 0% to 30% as still another embodiment.

[0018]

The oxidized form is a chemically modified substance in which part of the sequence of the antibody molecule is oxidized. A measuring method of the oxidized form is not particularly limited, so long as the oxidized form can be measured. The measuring method includes, for example, hydrophobic interaction chromatography, ion exchange chromatography, and the like. The amount of the oxidized forms is defined as 0% to 70% as one embodiment, and 0% to 50% as another embodiment.

[0019]

The degradant is a fragment generated by elimination of part of the antibody molecule. A measuring method of the degradant is not particularly limited, so long as the degradant can be measured. The measuring method includes, for example, size exclusion chromatography, gel electrophoresis, capillary electrophoresis, dynamic light scattering, light obscuration particle counting, micro flow imaging, and the like. The amount of the degradants is defined as 0% to 10% as one embodiment, and 0% to 5% as another embodiment.

[0020]

The multimer is a complex produced by collecting a plurality of antibody molecules. A measuring method of the multimer is not particularly limited, so long as the multimer can be measured. The measuring method includes, for example, size exclusion chromatography, gel electrophoresis, capillary electrophoresis, dynamic light scattering, light obscuration particle counting, micro flow imaging, and the like. The amount of the multimers is defined as 0% to 10% as one embodiment, and 0% to 5% as another embodiment.

[0021]

The term “stable” as used herein means that the amount of the impurities is suppressed at least for 6 months, preferably for 1 year, more preferably for 2 years at refrigerating temperature (2˚C to 8˚C); at least for 3 months, preferably for 6 months, more preferably for 1 year at room temperature (22˚C to 28˚C); or at least 1 week, preferably for 2 weeks at 40˚C. For example, the amount of the multimers and the amount of the degradants after storage at 5˚C for 2 years are each 10% or less, preferably 5% or less, more preferably 3% or less; the amount of the multimers and the amount of the degradants after storage at 25˚C for 3 months are each 10% or less, preferably 5% or less, more preferably 3% or less; or the amount of the multimers and the amount of the degradants after storage at 40˚C for 1 week are each 5% or less, preferably 3% or less.

[0022]

The term “about” as used herein means, when it is used in connection with numerical variables, a larger variable value, in general, within an experimental error (for example, within the 95% confidence interval for the mean), or within ±10% of the indicated value, and all the values of the variable. In this connection, even in the case of a numerical value without “about”, this interpretation shall be made.

[0023]

There are five classes of antibodies: IgG, IgM, IgA, IgD, and IgE. The basic structure of an antibody molecule is common to each class and is composed of a heavy chain having a molecular weight of 50,000 to 70,000 and a light chain of 20,000 to 30,000. The heavy chain usually consists of a polypeptide containing about 440 amino acids, and has a characteristic structure for each class, and the heavy chains of IgG, IgM, IgA, IgD, and IgE are called Igγ, Igμ, Igα, Igδ, and Igε, respectively. IgG further has subclasses: IgG1, IgG2, IgG3, and IgG4, and the heavy chains thereof are called Igγ1, Igγ2, Igγ3, and Igγ4, respectively. The light chain usually consists of a polypeptide containing about 220 amino acids, and two types of light chains, L type and K type, are known, and called Igλ and Igκ, respectively. With respect to the polypeptide constitution of the basic structure of an antibody molecule, two homologous heavy chains and two homologous light chains are linked by disulfide bonds (S-S bond) and noncovalent bonds, and the molecular weight is 150,000 to 190,000. Two types of light chains can pair with any heavy chain. An individual antibody molecule always consists of two identical light

chains and two identical heavy chains.

[0024]

There are four (five for the μ or ε chain) intrachain S-S bonds and two intrachain S-S bonds, and one loop is formed every 100 to 110 amino acid residues, and this steric structure is similar between each loop and is called a structural unit or a domain. The domain located at the amino terminus (N-terminus) for both heavy and light chains is not constant in its amino acid sequence, even if the antibody is a specimen that is the same class (subclass) from the same animal species, and the domain is called a variable region, and each domain is called a heavy chain variable region and a light chain variable region. The amino acid sequence at the carboxy terminal (C-terminal) side from the variable region is almost constant for each class or subclass, and is called a constant region.

[0025]

It is known that when an antibody is expressed in cells, the antibody undergoes a post-translational modification. As examples of the post-translational modification in which an amino acid residue is changed, cleavage of lysine at the C-terminus of a heavy chain by carboxypeptidase, modification to pyroglutamic acid by pyroglutamylation of glutamine or glutamic acid at the N-terminus of heavy and light chains, or the like, may be exemplified, and it is known that lysine at the C-terminus of a heavy chain is deleted, and modification to pyroglutamic acid occurs for most of the glutamine at the N-terminus of a heavy chain, in various antibodies (Journal of Pharmaceutical Sciences, 2008, Vol. 97, p. 2426). Further, it is known in the art that such a post-translational modification by pyroglutamylation at the N-terminus or lysine deletion at the C-terminus does not affect the antibody activity (Analytical Biochemistry, 2006, Vol. 348, p. 24-39).

[0026]

As an anti-human TSLP receptor antibody, the pharmaceutical composition of the present invention comprises the anti-human TSLP receptor antibody of the following (1) and/or (2):

(1) an anti-human TSLP receptor antibody comprising a heavy chain consisting of the amino acid sequence of SEQ ID NO: 1, and a light chain consisting of the amino acid sequence of SEQ ID NO: 3, and

(2) an anti-human TSLP receptor antibody consisting of amino acid sequences of an antibody generated by a post-translational modification of the anti-human TSLP receptor antibody comprising a heavy chain consisting of the amino acid sequence of SEQ ID NO: 1, and a light chain consisting of the amino acid sequence of SEQ ID NO: 3 (WO 2015/020193).

[0027]

In an embodiment, the post-translational modification in the anti-human TSLP receptor antibody of (2) is pyroglutamylation at the N-terminus of a heavy chain variable region and/or lysine deletion at the C-terminus of a heavy chain. For example, as the

anti-human TSLP receptor antibody of (2), an anti-human TSLP receptor antibody comprising a heavy chain consisting of the amino acid sequence of amino acids 1-447 of SEQ ID NO: 1, and a light chain consisting of the amino acid sequence of SEQ ID NO: 3 may be exemplified.

[0028]

In an embodiment, the pharmaceutical composition of the present invention comprises an anti-human TSLP receptor antibody comprising a heavy chain consisting of the amino acid sequence of SEQ ID NO: 1, and a light chain consisting of the amino acid sequence of SEQ ID NO: 3, and an anti-human TSLP receptor antibody comprising a heavy chain consisting of the amino acid sequence of amino acids 1-447 of SEQ ID NO: 1, and a light chain consisting of the amino acid sequence of SEQ ID NO: 3.

[0029]

The anti-human TSLP receptor antibody used in the present invention can be easily prepared by those skilled in the art, based on the sequence information of the heavy chain and the light chain of the anti-human TSLP receptor antibody disclosed in the present specification, using methods known in the art. As a preparation method of the anti-human TSLP receptor antibody used in the present invention, a method disclosed in WO 2015/020193 may be exemplified.

[0030]

The amount of the antibody in one unit pharmaceutical composition (preparation) is, for example, 0.001 mg to 1000 mg as one embodiment, and 0.01 mg to 100 mg as another embodiment. Each lower limit and each upper limit can be arbitrarily combined as desired.

[0031]

When the pharmaceutical composition is in a solid state (for example, lyophilized preparation, spray-dried preparation, or the like), the amount of the antibody is, for example, 0.001 mg to 1000 mg as one embodiment, and 0.01 mg to 100 mg as one embodiment. Each lower limit and each upper limit can be arbitrarily combined as desired.

The liquid volume when dissolved at the time of use is, for example, 0.1 mL to 100 mL as an embodiment, and 1 mL to 10 mL as another embodiment. Each lower limit and each upper limit can be arbitrarily combined as desired.

[0032]

When the pharmaceutical composition is a liquid state (liquid preparation), the concentration of the antibody is, for example, 1 mg/mL to 300 mg/mL (about 0.007 mmol/L to 2 mmol/L) as an embodiment, 1 mg/mL to 200 mg/mL (about 0.007 mmol/L to 1 mmol/L) as one embodiment, 1 mg/mL to 100 mg/mL (about 0.007 mmol/L to 0.7 mmol/L) as still another embodiment, and 10 mg/mL to 50 mg/mL (about 0.07 mmol/L to 0.3 mmol/L) as still another embodiment. Each lower limit and each upper limit can be

arbitrarily combined as desired.

The dose is 0.001 mg to 1000 mg as one embodiment, and 0.01 mg to 100 mg as another embodiment. Each lower limit and each upper limit can be arbitrarily combined as desired.

[0033]

Indications include prevention and/or treatment of various diseases in which human TSLP and a human TSLP receptor are involved in disease pathology, for example, prevention and/or treatment of asthma.

[0034]

A “pharmaceutically acceptable buffer” used in the present invention is not particularly limited, so long as it is pharmaceutically acceptable, and in a solution state, the pH of the solution can be adjusted within the desired pH range.

[0035]

More particularly, the pH is, for example, 5 to 6 as one embodiment, and 5.0 to 6.0 as another embodiment. When the buffer is phosphoric acid or a salt thereof, the pH is preferably 5.5 to 5.7, and when the buffer is histidine or a salt thereof, the pH is preferably 5.3 to 6.0.

When the pharmaceutical composition is a liquid preparation, the pH is defined as the pH of the liquid preparation, and when the pharmaceutical composition is a lyophilized preparation or a spray-dried preparation, the pH is defined as the pH of a solution obtained by dissolving the preparation in water.

[0036]

As the buffer component, for example, phosphoric acid (
sodium or potassium), citric acid, acetic acid, succinic acid, histidine, ascorbic acid, glutamic acid, lactic acid, maleic acid, trometamol, gluconic acid, a pharmaceutically acceptable salt thereof, or the like, is included as one embodiment. Phosphoric acid, or a pharmaceutically acceptable salt thereof (sodium salt or potassium salt) is included as another embodiment. Sodium dihydrogenphosphate is included as still another embodiment.

One kind or two or more kinds of these buffer components can be appropriately used in appropriate amounts.

[0037]

The concentration of the buffer is not particularly limited, so long as the pH can be adjusted within the desired pH range. More particularly, it is, for example, 5 to 100 mmol/L as an embodiment, 5 to 70 mmol/L as another embodiment, and 5 to 50 mmol/L as still another embodiment.

[0038]

When the pharmaceutical composition is in a solution state (liquid preparation) dissolved with water for injection, the amount of the buffer is, for example, 0.1 to 100

mg/mL as an embodiment, and 0.1 to 50 mg/mL as another embodiment. When the pharmaceutical composition is in a solid state (lyophilized preparation or spray-dried preparation) by lyophilization or the like, for example, the amount after reconstitution with 1 mL of water for injection is 5 to 100 mmol/L as an embodiment, 5 to 70 mmol/L as another embodiment, and 5 to 50 mmol/L as still another embodiment.

[0039]

"Arginine or a pharmaceutically acceptable salt thereof" used in the present invention is not particularly limited, so long as it is pharmaceutically acceptable arginine or a salt thereof. The arginine or a salt thereof has a function of stabilizing it. For example, L-arginine or L-arginine hydrochloride is included.

[0040]

The amount of arginine or a pharmaceutically acceptable salt thereof is 150 mg/mL (about 700 mmol/L) or less (excluding no addition) as an embodiment, 100 mg/mL (about 500 mmol/L) or less (excluding no addition) as another embodiment, and 45 mg/mL (about 210 mmol/L) or less (excluding no addition). When the anti-human TSLP receptor antibody is 30 mg/mL (about 0.2 mmol/L), 30 mg/mL (about 140 mmol/L) is preferable in order to ensure isotonicity from the aspect of osmotic pressure. As the lower limit (excluding no addition), for example, 10 mg/mL (about 50 mmol/L) or more may be exemplified. Each lower limit and each upper limit can be arbitrarily combined as desired.

[0041]

When the pharmaceutical composition is in a solution state (liquid preparation) dissolved with water for injection, for example, per 1 mL, the amount is 150 mg or less (excluding no addition) as an embodiment, 100 mg or less (excluding no addition) as another embodiment, and 45 mg or less (excluding no addition) as still another embodiment, or when the pharmaceutical composition is in a solid state (lyophilized preparation) by lyophilization or the like, for example, after reconstitution with water for injection, the arginine concentration is 150 mg/mL (about 700 mmol/L) or less (excluding no addition) as an embodiment, 100 mg/mL (about 500 mmol/L) or less (excluding no addition) as another embodiment, and 45 mg/mL (about 210 mmol/L) or less (excluding no addition) as still another embodiment. When the anti-human TSLP receptor antibody is 30 mg/mL, about 140 mmol/L is preferable in order to ensure isotonicity from the aspect of osmotic pressure.

[0042]

The surfactant used in the present invention is not particularly limited, so long as it is pharmaceutically acceptable and has surface activity.

More particularly, for example, nonionic surfactants (for example, sorbitan fatty acid esters, such as sorbitan monocaprylate, sorbitan monolaurate, sorbitan monopalmitate, and the like; glycerin fatty acid esters, such as glycerol monocaprylate,

glycerol monomyristate, glycerol monostearate, and the like; polyglycerol fatty acid esters, such as decaglyceryl monostearate, decaglyceryl distearate, decaglyceryl monolinoleate, and the like; polyoxyethylene sorbitan fatty acid esters, such as polyoxyethylene sorbitan monolaurate, polyoxyethylene sorbitan monooleate, polyoxyethylene sorbitan monostearate, polyoxyethylene sorbitan monopalmitate, polyoxyethylene sorbitan trioleate, polyoxyethylene sorbitan tristearate, and the like; polyoxyethylene sorbitol fatty acid ester, such as polyoxyethylene sorbitol tetrastearate, polyoxyethylene sorbitol tetraoleate, and the like; polyoxyethylene glycerin fatty acid esters, such as polyoxyethylene glyceryl monostearate, and the like; polyethylene glycol fatty acid esters, such as polyethylene glycol distearate, and the like; polyoxyethylene alkyl ethers, such as polyoxyethylene lauryl ether, and the like; polyoxyethylene polyoxypropylene alkyl ethers, such as polyoxyethylene polyoxypropylene glycol ether, polyoxyethylene polyoxypropylene propyl ether, polyoxyethylene polyoxypropylene cetyl ether, and the like; polyoxyethylene alkylphenyl ethers, such as polyoxyethylene nonylphenyl ether, and the like; polyoxyethylene hydrogenated castor oil, such as polyoxyethylene castor oil, polyoxyethylene hydrogenated castor oil, and the like; polyoxyethylene beeswax derivatives, such as polyoxyethylene sorbitol beeswax, and the like; polyoxyethylene lanolin derivatives, such as polyoxyethylene lanolin, and the like; and surfactants having an HLB of 6 to 18, such as polyoxyethylene fatty acid amides, and the like (for example, polyoxyethylene octadecanamide, and the like)); anionic surfactants (for example, alkyl sulfates having a C10-C18 alkyl group, such as sodium cetyl sulfate, sodium lauryl sulfate, sodium oleyl sulfate, and the like; polyoxyethylene alkyl ether sulfate in which the average number of moles of added ethylene oxide units is 2 to 4 and the number of carbon atoms of the alkyl group is 10 to 18, such as sodium polyoxyethylene lauryl sulfate, and the like; alkyl sulfosuccinate having C8-C18 alkyl group, such as sodium lauryl sulfosuccinate, and the like); natural surfactants, such as lecithin, glycerophospholipid, and the like; sphingophospholipids, such as sphingomyelin, and the like; and sucrose esters of C12-C18 fatty acids, are included.

One kind or two or more kinds of these surfactants can be appropriately selected and used.

[0043]

The surfactant is polyoxyethylene sorbitan fatty acid esters or polyoxyethylene polyoxypropylene alkyl ethers as an embodiment, polysorbates (for example, 20, 21, 40, 60, 65, 80, 81, or 85) or pluronic-type surfactants as another embodiment, polysorbates (for example, 20 or 80) or poloxamer 188 (pluronic F68) as still another embodiment, and is polysorbate 20 or polysorbate 80 as still another embodiment.

[0044]

The amount of the surfactant is 0.001 to 1% (w/v) as an embodiment, 0.005 to 0.5% (w/v) as another embodiment, and 0.01 to 0.2% (w/v) as still another embodiment.

Each lower limit and each upper limit can be arbitrarily combined as desired.

[0045]

The pharmaceutical composition of the present invention can be provided as a parenteral pharmaceutical composition, such as a liquid preparation by filling a container with the solution, or a lyophilized preparation, a spray-dried preparation, or the like obtained by subjecting the solution to lyophilization or spray-drying. The preferred pharmaceutical composition is a liquid preparation.

[0046]

To the pharmaceutical composition of the present invention, pharmaceutical additives, such as a suspending agent, a solubilizing agent, a tonicity agent, a preserving agent, an adsorption inhibitor, a diluting agent, a soothing agent, a sulfur-containing reducing agent, an antioxidant agent, or the like, can be appropriately added, if desired.

[0047]

As the suspending agent, for example, methyl cellulose, hydroxyethyl cellulose, gum arabic, tragacanth powder, sodium carboxymethyl cellulose, polyoxyethylene sorbitan monolaurate, and the like, may be exemplified.

[0048]

As the solubilization agent, for example, polyoxyethylene hydrogenated castor oil, nicotinamide, polyoxyethylene sorbitan monolaurate, macrogol, castor oil fatty acid ethyl ester, and the like, may be exemplified.

[0049]

As the tonicity agent, for example, sodium chloride, potassium chloride, calcium chloride, and the like, may be exemplified.

[0050]

As the preserving agent, for example, methyl parahydroxybenzoate, ethyl parahydroxybenzoate, sorbic acid, phenol, cresol, chlorocresol, benzyl alcohol, and the like, may be exemplified.

[0051]

As the adsorption inhibitor, for example, human serum albumin, lecithin, dextran, an ethylene oxide/propylene oxide copolymer, hydroxypropyl cellulose, methyl cellulose, polyoxyethylene hydrogenated castor oil, polyethylene glycol, and the like, may be exemplified.

[0052]

As the diluting agent, for example, sodium citrate hydrate, xylitol, and the like, may be exemplified.

[0053]

As the soothing agent, for example, inositol, lidocaine, and the like, may be exemplified.

[0054]

As the sulfur-containing reducing agent, for example, N-acetyl cysteine, N-acetyl homocysteine, thioctic acid, thiodiglycol, thioethanolamine, thioglycerol, thiosorbitol, thioglycolic acid and a salt thereof, sodium thiosulfate, glutathione, a compound having a sulfhydryl group, such as a thioalkanoic acid having 1 to 7 carbon atoms, and the like, may be exemplified.

[0055]

As the antioxidant agent, for example, erythorbic acid, dibutylhydroxytoluene, butylhydroxyanisole, α-tocopherol, tocopherol acetate, L-ascorbic acid and a salt thereof, L-ascorbyl palmitate, L-ascorbic acid stearate, sodium bisulfite, sodium sulfite, triamyl gallate, propyl gallate, or chelating agents, such as disodium ethylenediaminetetraacetate (EDTA), sodium pyrophosphate, sodium metaphosphate, and the like, may be exemplified.

[0056]

These pharmaceutical additives can be used in an appropriate amount within a range of an amount capable of acheiving the desired effect of the present invention.

[0057]

The method of producing the pharmaceutical composition of the present invention is characterized by comprising the anti-human TSLP receptor antibody, and arginine or a pharmaceutically acceptable salt thereof. Further, with respect to a composition containing other components, a method of producing a stable pharmaceutical composition by a known production method per se, comprising the anti-human TSLP receptor antibody, fully human type T7-27, is included.

[0058]

The container, which is filled with the pharmaceutical composition of the present invention, may be selected in accordance with the purpose of use. The container includes ones having a form of a specified capacity, such as a vial, an ampoule, and a syringe, or ones with a large capacity, such as a bottle. The container includes a syringe (including a disposable syringe) as an embodiment. By filling the syringe with a solution in advance and providing it as a prefilled syringe solution preparation, an operation such as a dissolution operation or the like becomes unnecessary in the medical field, a prompt response is expected.

[0059]

With respect to the material of the container, glass, plastics, or the like, may be exemplified. With respect to the surface treatment in the container, a silicone coating treatment, a sulfur treatment, a various low alkali treatment, and the like, may be carried out. By applying these treatments, it is expected to provide a more stable pharmaceutical composition.

EXAMPLES

[0060]

The present invention will now be further illustrated by, but is by no means limited to, the following Comparative Examples, Examples, and Experimental Examples.

The anti-human TSLP receptor antibody used in the Comparative Examples, Examples, and Experimental Examples was prepared by the method described in WO 2015/020193 or a similar method, and the concrete preparation procedure is shown in the Referential Example.

The symbol "-" in Tables means "with no additive".

[0061]

<<Referential Example: Preparation of fully human type anti-human TSLP receptor antibody, fully human type T7-27>>

With respect to fully human type T7-27 (hereinafter sometimes referred to as antibody A), which was a fully human type anti-human TSLP receptor antibody used in the Examples, the nucleotide sequence encoding the heavy chain is shown in SEQ ID NO: 2, the amino acid sequence encoded thereby is shown in SEQ ID NO: 1, the nucleotide sequence encoding the light chain is shown in SEQ ID NO: 4, and the amino acid sequence encoded thereby is shown in SEQ ID NO: 3.

[0062]

In accordance with WO 2015/020193, a GS vector (Lonza) into which both genes for the heavy chain and the light chain of antibody A were inserted was constructed. CHOK1SV cells (Lonza) were transfected with the vector to obtain a stable expression strain of the antibody, and the antibody was expressed. The culture supernatant was purified by a protein A column (GE Healthcare Japan) and an ion exchange chromatography to obtain a purified antibody of the fully human type antibody. As a result of analyzing the amino acid modification of the purified antibody A, it was assumed that lysine deletion at the C-terminal of the heavy chain occurred in most of the purified antibody.

[0063]

<<Example 1: Stabilization effect by selection of optimum pH>>

For liquid preparations containing antibody A, the influence of pH on stabilization of the preparations was evaluated.

[0064]

In this study, in order to evaluate the effect of pH, evaluation samples of sample Nos. A1 to A5 were prepared. The formulation of each evaluation sample is shown in Table 1-1 below.

[0065]

[Table 1-1]

Component	Function	A1 (/1mL)	A2 (/1mL)	A3 (/1mL)	A4 (/1mL)	A5 (/1mL)
Antibody A	Active ingredient	10 mg [approx. 0.07 mM]	10 mg [approx. 0.07 mM]	10 mg [approx. 0.07 mM]	10 mg [approx. 0.07 mM]	10 mg [approx. 0.07 mM]
Citric acid	Buffer	3.84 mg [20 mM]	3.84 mg [20 mM]	3.84 mg [20 mM]	—	—
$Na_2HPO_4 \cdot 12H_2O$	Buffer	—	—	—	7.16 mg [20 mM]	7.16 mg [20 mM]
L-Arginine· HCl	Stabilizer	29.49 mg [140 mM]	29.49 mg [140 mM]	29.49 mg [140 mM]	29.49 mg [140 mM]	29.49 mg [140 mM]
Polysorbate 80	Surfactant	0.20 mg [0.15 mM] (0.02 w/v%)	0.20 mg [0.15 mM] (0.02 w/v%)	0.20 mg [0.15 mM] (0.02 w/v%)	0.20 mg [0.15 mM] (0.02 w/v%)	0.20 mg [0.15 mM] (0.02 w/v%)
HCl	pH adjustor	adjusted to pH 4.0	adjusted to pH 5.0	adjusted to pH 6.0	adjusted to pH 6.0	adjusted to pH 7.0
NaOH	pH adjustor					

[0066]

In order to evaluate the stability of the liquid preparations, a thermal acceleration test (storage at 40˚C for 2 weeks and at 25˚C for 4 weeks) of each sample was performed. The quality of the antibody before and after the thermal acceleration was evaluated by size exclusion chromatography (SEC) and ion exchange chromatography (IEC). The analysis conditions are as follows.

[0067]

[Size exclusion chromatography (SEC)]

A G3000 SWXL column for SEC (Tosoh) was connected to an HPLC system, and a mobile phase consisting of 10 mmol/L phosphoric acid and 500 mmol/L NaCl pH 6.8 was flowed at a flow rate of 0.5 mL/min. Each sample was injected in an amount of 50 μg in terms of protein (for example, 5.0 μL in the case of 10 mg/mL). The analysis time was 30 minutes, and the detection was carried out at UV 280 nm. The column temperature was set to 30˚C, and the sample temperature was set to 5˚C.

[0068]

[Ion exchange chromatography (IEC)]

A Propac WCX 10 column for IEC (Dionex) was connected to an HPLC system. A mobile phase consisting of 20 mmol/L MES pH 6.0 was connected to a mobile phase A line, and a mobile phase consisting of 20 mmol/L MES and 500 mmol/L NaCl pH 6.0 was connected to a mobile phase B line, and the mobile phases were flowed at a flow rate of 1 mL/min. The samples were diluted to 1 mg/mL with the mobile phase A, and 10 μL was injected. The IEC gradient program of Table 1-2 was applied. The detection was carried out at UV 280 nm. The column temperature was set to 40˚C, and the sample temperature was set to 5˚C.

[0069]

[Table 1-2]

Time (min.)	Mobile phase B %
0	15
5	15
55	30
55.1	100
65	100
65.1	15
80	15

[0070]

The areas of multimer peaks and degradant peaks detected by SEC and the area of the main peak detected by IEC were measured by an automatic analysis method to determine the amounts (%) thereof. The amounts are defined as percentage (%), by measuring the area of multimer peaks or degradant peaks detected by SEC by an automatic analysis method and dividing it by the sum of all peak areas including the main peak, or by measuring the area of the main peak detected by IEC by an automatic analysis method and dividing it by the sum of all peak areas other than the main peak. The main peak as used herein means the peak of the active body.

[0071]

The evaluation results of SEC and IEC obtained in this Example are shown in Figures 1-1, 1-2, and 1-3. With respect to the SEC multimers, except for the storage at 40˚C for 2 weeks, the pH tended to increase them, particularly at a high pH (Figure 1-1). On the other hand, with respect to the SEC degradants, the pH tended to increase them, particularly at a low pH (Figure 1-2). With respect to the IEC main peak, the decrease was the smallest around pH 5-6, and it was the most stable (Figure 1-3). The above results were comprehensively judged, and it was confirmed that the optimum pH was around pH 5-6.

[0072]

<<Example 2: Suppression effect of arginine on increased multimers>>

For liquid preparations containing antibody A, the suppression effect of arginine on increased multimers was evaluated.

[0073]

In this study, sample Nos. B1 to B4 with different amounts of arginine added were prepared. The formulation of each evaluation sample is shown in Table 2-1 below.

[0074]

[Table 2-1]

Component	Function	B1 (/1mL)	B2 (/1mL)	B3 (/1mL)	B4 (/1mL)
Antibody A	Active ingredient	10 mg [approx. 0.07 mM]	10 mg [approx. 0.07 mM]	10 mg [approx. 0.07 mM]	10 mg [approx. 0.07 mM]
L-Histidine	Buffer	3.10 mg [20 mM]	3.10 mg [20 mM]	3.10 mg [20 mM]	3.10 mg [20 mM]
L-Arginine·HCl	Stabilizer	—	29.49 mg [140 mM]	44.24 mg [210 mM]	58.98 mg [280 mM]
HCl	pH adjustor	adjusted to pH 6.0	adjusted to pH 6.0	adjusted to pH 6.0	adjusted to pH 6.0
NaOH	pH adjustor				

[0075]

Each prepared sample listed in Table 2-1 was concentrated using spin columns to prepare samples B1-2 to B1-4, B2-2 to B2-4, B3-2 to B3-4, and B4-2 to B4-4. The formulation of each sample is shown in Table 2-2 below.

[0076]

[Table 2-2]

Component	Function	B1-2 (/1mL)	B2-2 (/1mL)	B3-2 (/1mL)	B4-2 (/1mL)
Antibody A	Active ingredient	120 mg [approx. 0.8 mM]	77 mg [approx. 0.5 mM]	74 mg [approx. 0.5 mM]	67 mg [approx. 0.4 mM]
L-Histidine	Buffer	3.10 mg [20 mM]	3.10 mg [20 mM]	3.10 mg [20 mM]	3.10 mg [20 mM]
L-Arginine·HCl	Stabilizer	—	29.49 mg [140 mM]	44.24 mg [210 mM]	58.98 mg [280 mM]

Component	Function	B1-3 (/1mL)	B2-3 (/1mL)	B3-3 (/1mL)	B4-3 (/1mL)
Antibody A	Active ingredient	189 mg [approx. 1.3 mM]	151 mg [approx. 1.0 mM]	161 mg [approx. 1.1 mM]	144 mg [approx. 1.0 mM]
L-Histidine	Buffer	3.10 mg [20 mM]	3.10 mg [20 mM]	3.10 mg [20 mM]	3.10 mg [20 mM]
L-Arginine·HCl	Stabilizer	—	29.49 mg [140 mM]	44.24 mg [210 mM]	58.98 mg [280 mM]

Component	Function	B1-4 (/1mL)	B2-4 (/1mL)	B3-4 (/1mL)	B4-4 (/1mL)
Antibody A	Active ingredient	231 mg [approx. 1.5 mM]	221 mg [approx. 1.5 mM]	210 mg [approx. 1.4 mM]	195 mg [approx. 1.3 mM]
L-Histidine	Buffer	3.10 mg [20 mM]	3.10 mg [20 mM]	3.10 mg [20 mM]	3.10 mg [20 mM]
L-Arginine·HCl	Stabilizer	—	29.49 mg [140 mM]	44.24 mg [210 mM]	58.98 mg [280 mM]

[0077]

<<Comparative Example 1>>

Sample Nos. B5 to B8 containing nicotinamide, mannitol, trehalose, or glycine were prepared. The formulation of each evaluation sample is shown in Table 2-3 below.

[0078]

[Table 2-3]

Component	Function	B5 (/1mL)	B6 (/1mL)	B7 (/1mL)	B8 (/1mL)
Antibody A	Active ingredient	10 mg [approx. 0.07 mM]	10 mg [approx. 0.07 mM]	10 mg [approx. 0.07 mM]	10 mg [approx. 0.07 mM]
L-Histidine	Buffer	3.10 mg [20 mM]	3.10 mg [20 mM]	3.10 mg [20 mM]	3.10 mg [20 mM]
Nicotinamide	Stabilizer	17.10 mg [140 mM]	—	—	—
Mannitol	Stabilizer	—	43.72 mg [240 mM]	—	—
Trehalose ($2H_2O$)	Stabilizer	—	—	90.80 mg [240 mM]	—
Glycine	Stabilizer	—	—	—	10.51 mg [140 mM]
HCl	pH adjustor	adjusted to pH 6.0	adjusted to pH 6.0	adjusted to pH 6.0	adjusted to pH 6.0
NaOH	pH adjustor				

[0079]

Each prepared sample listed in Table 2-3 was concentrated using spin columns to prepare samples B5-2 to B5-4, B6-2 to B6-4, B7-2 to B7-4, and B8-2 to B8-4. The formulation of each sample is shown in Table 2-4 below.

[0080]

[Table 2-4]

Component	Function	B5-2 (/1mL)	B6-2 (/1mL)	B7-2 (/1mL)	B8-2 (/1mL)
Antibody A	Active ingredient	149 mg [approx. 1.0 mM]	97 mg [approx. 0.6 mM]	77 mg [approx. 0.5 mM]	101 mg [approx. 0.7 mM]
L-Histidine	Buffer	3.10 mg [20 mM]	3.10 mg [20 mM]	3.10 mg [20 mM]	3.10 mg [20 mM]
Nicotinamide	Stabilizer	17.10 mg [140 mM]	—	—	—
Mannitol	Stabilizer	—	43.72 mg [240 mM]	—	—
Trehalose ($2H_2O$)	Stabilizer	—	—	90.80 mg [240 mM]	—
Glycine	Stabilizer	—	—	—	10.51 mg [140 mM]

Component	Function	B5-3 (/1mL)	B6-3 (/1mL)	B7-3 (/1mL)	B8-3 (/1mL)
Antibody A	Active ingredient	195 mg [approx. 1.3 mM]	161 mg [approx. 1.1 mM]	151 mg [approx. 1.0 mM]	191 mg [approx. 1.3 mM]
L-Histidine	Buffer	3.10 mg [20 mM]	3.10 mg [20 mM]	3.10 mg [20 mM]	3.10 mg [20 mM]
Nicotinamide	Stabilizer	17.10 mg [140 mM]	—	—	—
Mannitol	Stabilizer	—	43.72 mg [240 mM]	—	—
Trehalose ($2H_2O$)	Stabilizer	—	—	90.80 mg [240 mM]	—
Glycine	Stabilizer	—	—	—	10.51 mg [140 mM]

Component	Function	B5-4 (/1mL)	B6-4 (/1mL)	B7-4 (/1mL)	B8-4 (/1mL)
Antibody A	Active ingredient	212 mg [approx. 1.4 mM]	192 mg [approx. 1.3 mM]	170 mg [approx. 1.1 mM]	204 mg [approx. 1.4 mM]
L-Histidine	Buffer	3.10 mg [20 mM]	3.10 mg [20 mM]	3.10 mg [20 mM]	3.10 mg [20 mM]
Nicotinamide	Stabilizer	17.10 mg [140 mM]	—	—	—
Mannitol	Stabilizer	—	43.72 mg [240 mM]	—	—
Trehalose ($2H_2O$)	Stabilizer	—	—	90.80 mg [240 mM]	—
Glycine	Stabilizer	—	—	—	10.51 mg [140 mM]

[0081]

Each of these samples was evaluated by size exclusion chromatography (SEC). The analysis conditions are as follows.

[0082]

[Size exclusion chromatography (SEC)]

A G3000 SWXL column for SEC (Tosoh) was connected to an HPLC system, and a mobile phase consisting of 10 mmol/L phosphoric acid and 500 mmol/L NaCl pH 6.8 was flowed at a flow rate of 0.5 mL/min. Each sample was injected in an amount of 100 μg in terms of protein for the study of concentration. The analysis time was 30 minutes, and the detection was carried out at UV 280 nm. The column temperature was set to 30˚C, and the sample temperature was set to 20˚C.

[0083]

The area of multimer peaks detected by SEC was measured by an automatic analysis method to determine the amount (%) thereof. The amount is defined as percentage (%) by measuring the area of multimer peaks detected by SEC, and dividing it by the sum of all peak areas including the main peak. The main peak as used herein means the peak of the active body.

[0084]

The evaluation results (increase of multimer peaks %) of SEC obtained in this Example and Comparative Example are shown in Figure 2. In the preparations containing arginine (B2, B2-2 to B2-4, B3, B3-2 to B3-4, B4, and B4-2 to B4-4), an increase in multimers accompanying the increase in the concentration of the active ingredient was remarkably suppressed. On the other hand, in the preparations without arginine (B1, and B1-2 to B1-4) and the preparations with nicotinamide, mannitol, trehalose, or glycine (B5, B5-2 to B5-4, B6, B6-2 to B6-4, B7, B7-2 to B7-4, B8, and B8-2 to B8-4), an increase in multimers accompanying the increase in the concentration of the active ingredient was not suppressed. It was possible to confirm the suppression effect of arginine on increased multimers when the concentration of the active ingredient was increased.

[0085]

The difference in arginine concentration did not affect the suppression effect on increased multimers (B2, B2-2 to B2-4, B3, B3-2 to B3-4, B4, and B4-2 to B4-4).

[0086]

<<Example 3: Promoting effect of arginine, histidine, and pH on increased HIC hydrophilic peak>>

For liquid preparations containing antibody A, the influence of arginine, histidine, and pH on an increased HIC hydrophilic peak was evaluated.

[0087]

In this study, in order to evaluate the influence of arginine, histidine, and pH,

evaluation samples of sample Nos. C1 to C9 were prepared. The formulation of each evaluation sample is shown in Table 3-1 below.

[0088]

[Table 3-1]

Component	Function	C1 (/1mL)	C2 (/1mL)	C3 (/1mL)	C4 (/1mL)	C5 (/1mL)
Antibody A	Active ingredient	10 mg [approx. 0.07 mM]	10 mg [approx. 0.07 mM]	10 mg [approx. 0.07 mM]	10 mg [approx. 0.07 mM]	10 mg [approx. 0.07 mM]
$Na_2HPO_4 \cdot 12H_2O$	Buffer	—	—	7.16 mg [20 mM]	7.16 mg [20 mM]	7.16 mg [20 mM]
L-Histidine	Buffer	3.10 mg [20 mM]	3.10 mg [20 mM]	—	—	—
Citric acid	Buffer	—	—	—	—	—
L-Arginine· HCl	Stabilizer	—	29.49 mg [140 mM]	—	29.49 mg [140 mM]	—
Sorbitol	Stabilizer	—	—	—	—	—
Polysorbate 80	Surfactant	—	0.20 mg [0.15 mM] (0.02 w/v%)	—	0.20 mg [0.15 mM] (0.02 w/v%)	—
HCl	pH adjustor	adjusted to pH 6.0	adjusted to pH 6.0	adjusted to pH 6.0	adjusted to pH 6.0	adjusted to pH 7.0
NaOH	pH adjustor					

Component	Function	C6 (/1mL)	C7 (/1mL)	C8 (/1mL)	C9 (/1mL)
Antibody A	Active ingredient	10 mg [approx. 0.07 mM]	10 mg [approx. 0.07 mM]	10 mg [approx. 0.07 mM]	10 mg [approx. 0.07 mM]
$Na_2HPO_4 \cdot 12H_2O$	Buffer	7.16 mg [20 mM]	—	—	7.16 mg [20 mM]
L-Histidine	Buffer	—	—	—	—
Citric acid	Buffer	—	3.84 mg [20 mM]	3.84 mg [20 mM]	—
L-Arginine· HCl	Stabilizer	29.49 mg [140 mM]	29.49 mg [140 mM]	—	—
Sorbitol	Stabilizer	—	—	43.72 mg [240 mM]	43.72 mg [240 mM]
Polysorbate 80	Surfactant	0.20 mg [0.15 mM] (0.02 w/v%)	0.20 mg [0.15 mM] (0.02 w/v%)	0.20 mg [0.15 mM] (0.02 w/v%)	0.20 mg [0.15 mM] (0.02 w/v%)
HCl	pH adjustor	adjusted to pH 7.0	adjusted to pH 6.0	adjusted to pH 6.0	adjusted to pH 6.0
NaOH	pH adjustor				

[0089]

In order to evaluate the stability of the liquid preparations, a storage stability test (storage at 5˚C for 5 months) of each sample was performed. The quality of the antibody after the storage was evaluated by hydrophobic interaction chromatography (HIC). The analysis conditions are as follows.

[0090]

[Hydrophobic interaction chromatography (HIC)]

Two ProPac HIC-10 columns for HIC (Dionex) were connected to an HPLC system. A mobile phase consisting of 800 mmol/L ammonium sulfate, 20 mmol/L sodium phosphate pH7.0 was connected to a mobile phase A line, and a mobile phase consisting of 20 mmol/L sodium phosphate pH7.0 was connected to a mobile phase B line, and the mobile phases were flowed at a flow rate of 0.8 mL/min. The samples were diluted to 1 mg/mL with the mobile phase A, and 50 μL was injected. The HIC gradient program of Table 3-2 was applied. The detection was carried out at UV 280 nm. The column temperature was set to 30˚C, and the sample temperature was set to 25˚C.

[0091]

[Table 3-2]

Time (min.)	Mobile phase B %
0	0
2	0
47	20
49	20
54	100
56	0
66	0

[0092]

The area of a hydrophilic peak detected by HIC, as an index of an oxidized form, was measured by an automatic analysis method to determine the amount (%) thereof. The amount is defined as percentage (%), by measuring the area of a hydrophilic peak detected by HIC by an automatic integration method and dividing it by the sum of all peak areas including the main peak. The main peak as used herein means the peak of the active body.

[0093]

The evaluation results of HIC obtained in this Example are shown in Figure 3-3.

[0094]

[Table 3-3]

Sample No.	HIC hydrophilic peak (%)
C1	23.30
C2	26.40
C3	19.18
C4	23.84
C5	19.70
C6	40.72
C7	23.34
C8	21.07
C9	20.70

[0095]

In the preparations containing arginine (C2, C4, C6, and C7), a tendency to promote an increase in the HIC hydrophilic peak was observed in comparison with the preparations without arginine (C3 and C5) and preparations containing sorbitol (C8 and C9).

[0096]

In the preparation containing arginine of pH 7.0 (C6), a tendency to promote an increase in the HIC hydrophilic peak was observed in comparison with the preparation containing arginine of pH 6.0 (C4).

[0097]

In the preparation containing histidine (C1), a tendency to promote an increase in the HIC hydrophilic peak was observed in comparison with the preparation containing phosphoric acid (C3).

[0098]

<<Example 4: Study of preparations by design of experiments method>>

For liquid preparations containing antibody A, pH and arginine concentration were examined.

[0099]

In this study, in order to evaluate the effects of arginine and pH, evaluation samples of sample Nos. D1 to D18 were prepared in accordance with design of experiments method. The formulation of each evaluation sample is shown in Tables 4-1 and 4-2 below.

[0100]

[Table 4-1]

Component	Function	D1 (/1mL)	D2 (/1mL)	D3 (/1mL)	D4 (/1mL)	D5 (/1mL)
Antibody A	Active ingredient	10 mg [approx. 0.07 mM]	50 mg [approx. 0.3 mM]	10 mg [approx. 0.07 mM]	50 mg [approx. 0.3 mM]	10 mg [approx. 0.07 mM]
$Na_2HPO_4 \cdot 12H_2O$	Buffer	7.16 mg [20 mM]	7.16 mg [20 mM]	7.16 mg [20 mM]	7.16 mg [20 mM]	7.16 mg [20 mM]
L-Histidine	Buffer	—	—	—	—	—
L-Arginine·HCl	Stabilizer	58.98 mg [280 mM]	58.98 mg [280 mM]	—	—	58.98 mg [280 mM]
Polysorbate 80	Surfactant	—	—	0.40 mg [0.31 mM] (0.04 w/v%)	—	0.40 mg [0.31 mM] (0.04 w/v%)
HCl	pH adjustor	adjusted to pH 6.5	adjusted to pH 5.5	adjusted to pH 6.5	adjusted to pH 6.5	adjusted to pH 5.5

Component	Function	D6 (/1mL)	D7 (/1mL)	D8 (/1mL)	D9 (/1mL)
Antibody A	Active ingredient	50 mg [approx. 0.3 mM]	10 mg [approx. 0.07 mM]	50 mg [approx. 0.3 mM]	10 mg [approx. 0.07 mM]
$Na_2HPO_4 \cdot 12H_2O$	Buffer	7.16 mg [20 mM]	7.16 mg [20 mM]	7.16 mg [20 mM]	—
L-Histidine	Buffer	—	—	—	3.10 mg [20 mM]
L-Arginine·HCl	Stabilizer	—	—	58.98 mg [280 mM]	58.98 mg [280 mM]
Polysorbate 80	Surfactant	0.40 mg [0.31 mM] (0.04 w/v%)	—	0.40 mg [0.31 mM] (0.04 w/v%)	—
HCl	pH adjustor	adjusted to pH 5.5	adjusted to pH 5.5	adjusted to pH 6.5	adjusted to pH 6.0

[0101]

[Table 4-2]

Component	Function	D10 (/1mL)	D11 (/1mL)	D12 (/1mL)	D13 (/1mL)	D14 (/1mL)
Antibody A	Active ingredient	50 mg [approx. 0.3 mM]	10 mg [approx. 0.07 mM]	50 mg [approx. 0.3 mM]	10 mg [approx. 0.07 mM]	50 mg [approx. 0.3 mM]
$Na_2HPO_4 \cdot 12H_2O$	Buffer	—	—	—	—	—
L-Histidine	Buffer	3.10 mg [20 mM]	3.10 mg [20 mM]	3.10 mg [20 mM]	3.10 mg [20 mM]	3.10 mg [20 mM]
L-Arginine· HCl	Stabilizer	58.98 mg [280 mM]	—	—	58.98 mg [280 mM]	—
Polysorbate 80	Surfactant	—	0.40 mg [0.31 mM] (0.04 w/v%)	—	0.40 mg [0.31 mM] (0.04 w/v%)	0.40 mg [0.31 mM] (0.04 w/v%)
HCl	pH adjustor	adjusted to pH 5.0	adjusted to pH 6.0	adjusted to pH 6.0	adjusted to pH 5.0	adjusted to pH 5.0

Component	Function	D15 (/1mL)	D16 (/1mL)	D17 (/1mL)	D18 (/1mL)
Antibody A	Active ingredient	10 mg [approx. 0.07 mM]	50 mg [approx. 0.3 mM]	30 mg [approx. 0.2 mM]	30 mg [approx. 0.2 mM]
$Na_2HPO_4 \cdot 12H_2O$	Buffer	—	—	7.16 mg [20 mM]	—
L-Histidine	Buffer	3.10 mg [20 mM]	3.10 mg [20 mM]	—	3.10 mg [20 mM]
L-Arginine· HCl	Stabilizer	—	58.98 mg [280 mM]	29.49 mg [140 mM]	29.49 mg [140 mM]
Polysorbate 80	Surfactant	—	0.40 mg [0.31 mM] (0.04 w/v%)	0.20 mg [0.15 mM] (0.02 w/v%)	0.20 mg [0.15 mM] (0.02 w/v%)
HCl	pH adjustor	adjusted to pH 5.0	adjusted to pH 6.0	adjusted to pH 6.0	adjusted to pH 5.5

[0102]

In order to evaluate the stability of the liquid preparations, a thermal acceleration test (storage at 40˚C for 1 week) of each sample was performed. The purity of the antibody before and after the thermal acceleration was evaluated by size exclusion chromatography (SEC), ion exchange chromatography (IEC), and hydrophobic interaction chromatography (HIC). The analysis conditions are as follows.

[0103]

[Size exclusion chromatography (SEC)]

A G3000 SWXL column for SEC (Tosoh) was connected to an HPLC system, and a mobile phase consisting of 20 mmol/L phosphoric acid and 1 mol/L NaCl pH 6.5 was flowed at a flow rate of 0.5 mL/min. Each sample was injected in an amount of 50 μg in terms of protein. The analysis time was 40 minutes, and the detection was carried out at UV 280 nm. The column temperature was set to 30˚C, and the sample temperature was set to 5˚C.

[0104]

[Ion exchange chromatography (IEC)]

A Propac WCX 10 column for IEC (Dionex) was connected to an HPLC system. A mobile phase consisting of 25 mmol/L phosphoric acid pH 6.0 was connected to a mobile phase A line, and a mobile phase consisting of 25 mmol/L phosphoric acid and 500 mmol/L NaCl pH 6.0 was connected to a mobile phase B line, and the mobile phases were flowed at a flow rate of 1 mL/min. The samples were diluted to 1 mg/mL with the mobile phase A, and 10 μL was injected. The analysis time was 80 minutes, and the IEC gradient program of Table 4-3 was applied. The detection was carried out at UV 280 nm. The column temperature was set to 35˚C, and the sample temperature was set to 5˚C.

[0105]

[Table 4-3]

Time (min.)	Mobile phase B %
0	10
5	10
55	35
55.1	100
65	100
65.1	10
80	10

[0106]

[Hydrophobic interaction chromatography (HIC)]

Two ProPac HIC-10 columns for HIC (Dionex) were connected to an HPLC system. A mobile phase consisting of 800 mmol/L ammonium sulfate and 20 mmol/L sodium phosphate pH7.0 was connected to a mobile phase A line, and a mobile phase consisting of 20 mmol/L sodium phosphate pH7.0 was connected to a mobile phase B line, and the mobile phases were flowed at a flow rate of 0.8 mL/min. The samples were diluted to 1 mg/mL with the mobile phase A, and 50 μL was injected. The HIC gradient program of Table 4-4 was applied. The detection was carried out at UV 280 nm. The column temperature was set to 30˚C, and the sample temperature was set to 25˚C.

[0107]

[Table 4-4]

Time (min.)	Mobile phase B %
0	0
2	0
47	20
49	20
54	100
56	0
66	0

[0108]

The areas of multimer peaks and degradant peaks detected by SEC, the area of the main peak detected by IEC, and the area of a hydrophilic peak detected by HIC were measured by an automatic analysis method to determine the amounts (%) thereof. The amounts are defined as percentage (%), by measuring the area of multimer peaks or degradant peaks detected by SEC by an automatic analysis method and dividing it by the sum of all peak areas including the main peak, by measuring the area of the main peak detected by IEC by an automatic analysis method and dividing it by the sum of all peak areas other than the main peak, or by measuring the area of a hydrophilic peak detected by HIC by an automatic integration method and dividing it by the sum of all peak areas including the main peak. The main peak as used herein means the peak of the active body.

[0109]

The evaluation results of SEC, IEC, and HIC obtained in this Example are shown in Figure 4-5.

[0110]

[Table 4-5]

Sample No.	SEC Multimer %		SEC Degradant %		IEC Main peak %		HIC Hydrophilic peak %	
	Initial	40°C 1W	Initial	40°C 1W	Initial	40°C 1W	Initial	40°C 1W
D1	2.18	1.56	1.12	1.90	59.30	55.85	14.83	22.51
D2	2.06	1.40	1.11	2.07	60.34	57.98	15.14	17.76
D3	2.78	2.41	1.10	1.71	60.99	54.46	15.58	20.20
D4	3.41	3.88	1.07	1.68	60.15	55.16	15.05	17.38
D5	1.98	1.22	1.09	1.97	60.28	57.44	15.96	22.55
D6	2.68	2.37	1.10	1.78	60.41	56.11	15.64	17.08
D7	2.13	1.54	1.13	1.85	59.68	55.39	15.20	15.68
D8	2.40	1.92	1.11	1.89	60.07	55.42	15.81	28.49
D9	2.14	1.23	1.20	1.74	59.67	59.40	15.43	17.70
D10	1.92	1.17	1.36	3.16	59.90	57.08	15.42	16.57
D11	2.01	1.04	1.17	1.65	59.48	58.54	15.97	21.07
D12	2.35	1.67	1.15	1.73	60.04	56.96	15.26	17.65
D13	1.88	0.90	1.19	3.06	58.93	54.89	16.35	25.35
D14	2.09	1.23	1.29	2.22	59.18	54.72	15.79	17.35
D15	1.90	0.92	1.26	1.98	59.16	54.91	15.40	15.60
D16	2.20	1.51	1.18	1.92	59.03	57.39	15.96	21.46
D17	2.25	1.84	1.19	1.78	59.01	56.49	15.78	20.13
D18	2.12	1.31	1.17	2.35	58.97	56.16	15.77	18.02

[0111]

Statistical analysis of factor screening was conducted based on the results, and p-values indicating the degree of contribution of each index were calculated using design of experiments software, Design-Expert (a product of Stat-Ease, Inc.). The results are shown in Table 4-6.

[0112]

[Table 4-6]

Phosphate buffer formulation:

	SEC Multimer %	SEC Degradant % incerased	IEC Main peak % decreased	HIC Hydrophilic peak % increased
Antibody A concentration	0.0262	0.0996	0.0370	0.4252
Polysorbate 80 concentration	0.3347	0.1273	NA	0.1011
pH	0.0377	0.0025	0.0027	NA
Arginine concentration	0.8577	0.0044	0.0157	0.2734

Histidine buffer formulation:

	SEC Multimer %	SEC Degradant % incerased	IEC Main peak % decreased	HIC Hydrophilic peak % increased
Antibody A concentration	0.0262	0.0996	0.0370	0.4252
Polysorbate 80 concentration	0.3347	0.1273	NA	0.1011
pH	0.0377	0.0025	0.0027	NA
Arginine concentration	0.8577	0.0044	0.0157	0.2734

*NA: not assayed

[0113]

From the results, with respect to all the evaluation items of SEC, IEC, and HIC, as an index of stability, the range of maximizing the desirability for arginine concentration and pH, which contributed greatly to the stabilizing effect, was analyzed using design of experiments software, Design-Expert (a product of Stat-Ease, Inc.). The results are shown in Figure 3-1 (a group of phosphoric acid-containing formulations) and Figure 3-2 (a group of histidine-containing formulations).

[0114]

It was suggested from the results that the antibody was stable in the range of pH 5.5 to 5.7 and an arginine concentration range of 0 to 210 mmol/L (excluding no addition) in the phosphoric acid-containing formulations, and that the antibody was stable in the range of pH 5.3 to 6.0 and an arginine concentration range of 0 to 210 mmol/L (excluding no addition) in the histidine-containing formulations.

[0115]

<<Example 5: Suppression of generation of insoluble microparticles by surfactant>>

For liquid preparations containing antibody A, the suppression effect of surfactants on generation of insoluble microparticles after stress loading was evaluated.

[0116]

In this study, in order to evaluate the effects of polysorbate 80, polysorbate 20, and poloxamer 188 (Pluronic F68), which were surfactants, evaluation samples of sample Nos. E1 to E18 were prepared. The formulation of each evaluation sample is shown in Tables 5-1 and 5-2 below.

[0117]

[Table 5-1]

Component	Function	E1 (/1mL)	E2 (/1mL)	E3 (/1mL)	E4 (/1mL)	E5 (/1mL)
Antibody A	Active ingredient	10 mg [approx. 0.07 mM]	10 mg [approx. 0.07 mM]	10 mg [approx. 0.07 mM]	10 mg [approx. 0.07 mM]	10 mg [approx. 0.07 mM]
L-Histidine	Buffer	3.10 mg [20 mM]	3.10 mg [20 mM]	3.10 mg [20 mM]	3.10 mg [20 mM]	3.10 mg [20 mM]
L-Arginine· HCl	Stabilizer	29.49 mg [140 mM]	29.49 mg [140 mM]	29.49 mg [140 mM]	29.49 mg [140 mM]	29.49 mg [140 mM]
Polysorbate 80	Surfactant	—	0.20 mg [0.15 mM] (0.02 w/v%)	0.50 mg [0.38 mM] (0.05 w/v%)	1.00 mg [0.76 mM] (0.10 w/v%)	2.00 mg [1.53 mM] (0.20 w/v%)
Polysorbate 20	Surfactant	—	—	—	—	—
Poloxamer 188	Surfactant	—	—	—	—	—
HCl	pH adjustor	adjusted to pH 6.0	adjusted to pH 6.0	adjusted to pH 6.0	adjusted to pH 6.0	adjusted to pH 6.0

Component	Function	E6 (/1mL)	E7 (/1mL)	E8 (/1mL)	E9 (/1mL)
Antibody A	Active ingredient	30 mg [approx. 0.2 mM]	30 mg [approx. 0.2 mM]	30 mg [approx. 0.2 mM]	30 mg [approx. 0.2 mM]
L-Histidine	Buffer	3.10 mg [20 mM]	3.10 mg [20 mM]	3.10 mg [20 mM]	3.10 mg [20 mM]
L-Arginine· HCl	Stabilizer	29.49 mg [140 mM]	29.49 mg [140 mM]	29.49 mg [140 mM]	29.49 mg [140 mM]
Polysorbate 80	Surfactant	—	0.20 mg [0.15 mM] (0.02 w/v%)	0.50 mg [0.38 mM] (0.05 w/v%)	1.00 mg [0.76 mM] (0.10 w/v%)
Polysorbate 20	Surfactant	—	—	—	—
Poloxamer 188	Surfactant	—	—	—	—
HCl	pH adjustor	adjusted to pH 6.0	adjusted to pH 6.0	adjusted to pH 6.0	adjusted to pH 6.0

[0118]

[Table 5-2]

Component	Function	E10 (/1mL)	E11 (/1mL)	E12 (/1mL)	E13 (/1mL)	E14 (/1mL)
Antibody A	Active ingredient	30 mg [approx. 0.2 mM]	10 mg [approx. 0.07 mM]	30 mg [approx. 0.2 mM]	10 mg [approx. 0.07 mM]	10 mg [approx. 0.07 mM]
L-Histidine	Buffer	3.10 mg [20 mM]	3.10 mg [20 mM]	3.10 mg [20 mM]	3.10 mg [20 mM]	3.10 mg [20 mM]
L-Arginine· HCl	Stabilizer	29.49 mg [140 mM]	29.49 mg [140 mM]	29.49 mg [140 mM]	29.49 mg [140 mM]	29.49 mg [140 mM]
Polysorbate 80	Surfactant	2.00 mg [1.53 mM] (0.20 w/v%)	—	—	—	—
Polysorbate 20	Surfactant	—	0.20 mg [0.16 mM] (0.02 w/v%)	0.20 mg [0.16 mM] (0.02 w/v%)	—	—
Poloxamer 188	Surfactant	—	—	—	0.20 mg [0.02 mM] (0.02 w/v%)	0.50 mg [0.06 mM] (0.05 w/v%)
HCl	pH adjustor	adjusted to pH 6.0	adjusted to pH 6.0	adjusted to pH 6.0	adjusted to pH 6.0	adjusted to pH 6.0

Component	Function	E15 (/1mL)	E16 (/1mL)	E17 (/1mL)	E18 (/1mL)
Antibody A	Active ingredient	10 mg [approx. 0.07 mM]	30 mg [approx. 0.2 mM]	30 mg [approx. 0.2 mM]	30 mg [approx. 0.2 mM]
L-Histidine	Buffer	3.10 mg [20 mM]	3.10 mg [20 mM]	3.10 mg [20 mM]	3.10 mg [20 mM]
L-Arginine· HCl	Stabilizer	29.49 mg [140 mM]	29.49 mg [140 mM]	29.49 mg [140 mM]	29.49 mg [140 mM]
Polysorbate 80	Surfactant	—	—	—	—
Polysorbate 20	Surfactant	—	—	—	—
Poloxamer 188	Surfactant	1.00 mg [0.12 mM] (0.10 w/v%)	0.20 mg [0.02 mM] (0.02 w/v%)	0.50 mg [0.06 mM] (0.05 w/v%)	1.00 mg [0.12 mM] (0.10 w/v%)
HCl	pH adjustor	adjusted to pH 6.0	adjusted to pH 6.0	adjusted to pH 6.0	adjusted to pH 6.0

[0119]

In order to evaluate the stability of liquid preparations, each sample was subjected to a stress load test. For each sample, three freeze-thaw cycles between -80˚C and 5˚C were carried out, and samples were shaken at 150 rpm for 24 hours and stored under 1,000 lux for 24 hours. The number of insoluble microparticles in each sample after stress loading was evaluated by light obscuration particle counting.

[0120]

[Light obscuration particle counting]

After 0.7 mL of a sample was placed in a 1.5 mL plastic tube, it was degassed for 2 hours under conditions of 25˚C and 75 Torr using a vacuum dryer. After degassing, measurement was carried out using a HIAC liquid particle counter for laboratories, with

the setting of a tare volume of 0.2 mL, a sampling volume of 0.2 mL, and a Run cycle of 2 (the first measurement was discarded).

[0121]

In order to evaluate the influence of the surfactant concentration and the active ingredient concentration on the protein, with respect to samples E1 to E10, the samples were stored at 25˚C for 4 weeks, and the samples after storage were evaluated by hydrophobic interaction chromatography (HIC).

[0122]

[Hydrophobic interaction chromatography (HIC)]

Two ProPac HIC-10 columns for HIC (Dionex) were connected to an HPLC system. A mobile phase consisting of 800 mmol/L ammonium sulfate and 20 mmol/L sodium phosphate pH7.0 was connected to a mobile phase A line, and a mobile phase consisting of 20 mmol/L sodium phosphate pH7.0 was connected to a mobile phase B line, and the mobile phases were flowed at a flow rate of 0.8 mL/min. The samples were diluted to 1 mg/mL with the mobile phase A, and 50 μL was injected. The HIC gradient program of Table 5-3 was applied. The detection was carried out at UV 280 nm. The column temperature was set to 30˚C, and the sample temperature was set to 25˚C.

[0123]

[Table 5-3]

Time (min.)	Mobile phase B %
0	0
2	0
47	20
49	20
54	100
56	0
66	0

[0124]

The area of the hydrophilic peak detected by HIC was measured by an automatic analysis method to determine the amount (%) thereof. The amount is defined as percentage (%), by measuring the area of the hydrophilic peak detected by HIC by an automatic integration method and dividing it by the sum of all peak areas including the main peak. The main peak as used herein means the peak of the active body.

[0125]

The evaluation results of the numbers of insoluble microparticles obtained in this Example are shown in Table 5-4. Whether or not a surfactant is added, an increase in the number of insoluble microparticles after stress loading was observed, but the increase in the number of insoluble microparticles was suppressed in all samples added with a

surfactant.

[0126]

[Table 5-4]

Sample No.	Buffer/ Additive/ pH	Active ingredient concentration	Surfactant/ Concentration added	Before stress load (particles/mL)	After Stress load (particles/mL)
E1	20 mM Histidine/ 140 mM Arginine/ pH 6.0	10 mg/mL	Polysorbate 80/0%	190	23285
E2			Polysorbate 80/0.02%	195	3065
E3			Polysorbate 80/0.05%	105	640
E4			Polysorbate 80/0.10%	225	780
E5			Polysorbate 80/0.20%	140	935
E6		30 mg/mL	Polysorbate 80/0%	180	22145
E7			Polysorbate 80/0.02%	45	2480
E8			Polysorbate 80/0.05%	65	790
E9			Polysorbate 80/0.10%	65	720
E10			Polysorbate 80/0.20%	90	335
E11		10 mg/mL	Polysorbate 20/0.02%	505	990
E12		30 mg/mL	Polysorbate 20/0.02%	150	1190
E13		10 mg/mL	Poloxamer 188/0.02%	385	965
E14			Poloxamer 188/0.05%	115	1075
E15			Poloxamer 188/0.10%	125	845
E16		30 mg/mL	Poloxamer 188/0.02%	135	4630
E17			Poloxamer 188/0.05%	85	1500
E18			Poloxamer 188/0.10%	185	2750

[0127]

The evaluation results of HIC obtained in this Example are shown in Figures 4-1 and 4-2. For the samples containing polysorbate 80, an increasing tendency of the HIC hydrophilic peak was observed with increasing the concentration of polysorbate 80. On the other hand, for the samples containing the active ingredient at a concentration of 30 mg/mL (Figure 4-2), the increase in the HIC hydrophilic peak was suppressed in comparison with the samples containing the active ingredient at a concentration of 10 mg/mL (Figure 4-1).

[0128]

<<Example 6: Reduction of viscosity by arginine>>

For liquid preparations containing antibody A, the effect of reducing the viscosity by arginine was evaluated.

[0129]

In this study, in order to evaluate the effect of arginine on the viscosity of liquid preparations, evaluation samples of sample Nos. F1 to F12 were prepared. The formulation of each evaluation sample is shown in Table 6-1 below.

[0130]

[Table 6-1]

Component	Function	F1 (/1mL)	F2 (/1mL)	F3 (/1mL)	F4 (/1mL)	F5 (/1mL)
Antibody A	Active ingredient	150 mg [approx. 1.0 mM]	120 mg [approx. 0.8 mM]	90 mg [approx. 0.6 mM]	150 mg [approx. 1.0 mM]	120 mg [approx. 0.8 mM]
L-Histidine	Buffer	3.10 mg [20 mM]	3.10 mg [20 mM]	3.10 mg [20 mM]	—	—
$Na_2HPO_4 \cdot 12H_2O$	Buffer	—	—	—	7.16 mg [20 mM]	7.16 mg [20 mM]
Citric acid	Buffer	—	—	—	—	—
Succinic acid	Buffer	—	—	—	—	—
L-Arginine· HCl	Stabilizer	29.49 mg [140 mM]	29.49 mg [140 mM]	29.49 mg [140 mM]	29.49 mg [140 mM]	29.49 mg [140 mM]
HCl	pH adjustor	adjusted to pH 6.0	adjusted to pH 6.0	adjusted to pH 6.0	adjusted to pH 6.0	adjusted to pH 6.0
NaOH	pH adjustor					

Component	Function	F6 (/1mL)	F7 (/1mL)	F8 (/1mL)	F9 (/1mL)	F10 (/1mL)
Antibody A	Active ingredient	90 mg [approx. 0.6 mM]	150 mg [approx. 1.0 mM]	120 mg [approx. 0.8 mM]	90 mg [approx. 0.6 mM]	150 mg [approx. 1.0 mM]
L-Histidine	Buffer	—	—	—	—	—
$Na_2HPO_4 \cdot 12H_2O$	Buffer	7.16 mg [20 mM]	—	—	—	—
Citric acid	Buffer	—	3.84 mg [20 mM]	3.84 mg [20 mM]	3.84 mg [20 mM]	—
Succinic acid	Buffer	—	—	—	—	2.36 mg [20 mM]
L-Arginine· HCl	Stabilizer	29.49 mg [140 mM]	29.49 mg [140 mM]	29.49 mg [140 mM]	29.49 mg [140 mM]	29.49 mg [140 mM]
HCl	pH adjustor	adjusted to pH 6.0	adjusted to pH 6.0	adjusted to pH 6.0	adjusted to pH 6.0	adjusted to pH 6.0
NaOH	pH adjustor					

Component	Function	F11 (/1mL)	F12 (/1mL)
Antibody A	Active ingredient	120 mg [approx. 0.8 mM]	90 mg [approx. 0.6 mM]
L-HIstidine	Buffer	—	—
$Na_2HPO_4 \cdot 12H_2O$	Buffer	—	—
Citric acid	Buffer	—	—
Succinic acid	Buffer	2.36 mg [20 mM]	2.36 mg [20 mM]
L-Arginine· HCl	Stabilizer	29.49 mg [140 mM]	29.49 mg [140 mM]
HCl	pH adjustor	adjusted to pH 6.0	adjusted to pH 6.0
NaOH	pH adjustor		

[0131]

<<Comparative Example 2>>

In order to evaluate the effects of arginine and pH, evaluation samples without arginine (sample Nos. F13 to F27) and evaluation samples at a low pH (sample Nos. F19 to F21) were prepared. The formulation of each evaluation sample is shown in Table 6-2 below.

[0132]

[Table 6-2]

Component	Function	F13 (/1mL)	F14 (/1mL)	F15 (/1mL)	F16 (/1mL)	F17 (/1mL)
Antibody A	Active ingredient	150 mg [approx. 1.0 mM]	120 mg [approx. 0.8 mM]	90 mg [approx. 0.6 mM]	150 mg [approx. 1.0 mM]	120 mg [approx. 0.8 mM]
L-Histidine	Buffer	3.10 mg [20 mM]	3.10 mg [20 mM]	3.10 mg [20 mM]	—	—
$Na_2HPO_4 \cdot 12H_2O$	Buffer	—	—	—	7.16 mg [20 mM]	7.16 mg [20 mM]
Citric acid	Buffer	—	—	—	—	—
Succinic acid	Buffer	—	—	—	—	—
HCl	pH adjustor	adjusted to pH 6.0	adjusted to pH 6.0	adjusted to pH 6.0	adjusted to pH 6.0	adjusted to pH 6.0
NaOH	pH adjustor					

Component	Function	F18 (/1mL)	F19 (/1mL)	F20 (/1mL)	F21 (/1mL)	F22 (/1mL)
Antibody A	Active ingredient	90 mg [approx. 0.6 mM]	150 mg [approx. 1.0 mM]	120 mg [approx. 0.8 mM]	90 mg [approx. 0.6 mM]	150 mg [approx. 1.0 mM]
L-Histidine	Buffer	—	—	—	—	—
$Na_2HPO_4 \cdot 12H_2O$	Buffer	7.16 mg [20 mM]	—	—	—	—
Citric acid	Buffer	—	3.84 mg [20 mM]	3.84 mg [20 mM]	3.84 mg [20 mM]	3.84 mg [20 mM]
Succinic acid	Buffer	—	—	—	—	—
HCl	pH adjustor	adjusted to pH 6.0	adjusted to pH 5.0	adjusted to pH 5.0	adjusted to pH 5.0	adjusted to pH 6.0
NaOH	pH adjustor					

Component	Function	F23 (/1mL)	F24 (/1mL)	F25 (/1mL)	F26 (/1mL)	F27 (/1mL)
Antibody A	Active ingredient	120 mg [approx. 0.8 mM]	90 mg [approx. 0.6 mM]	150 mg [approx. 1.0 mM]	120 mg [approx. 0.8 mM]	90 mg [approx. 0.6 mM]
L-Histidine	Buffer	—	—	—	—	—
$Na_2HPO_4 \cdot 12H_2O$	Buffer	—	—	—	—	—
Citric acid	Buffer	3.84 mg [20 mM]	3.84 mg [20 mM]	—	—	—
Succinic acid	Buffer	—	—	2.36 mg [20 mM]	2.36 mg [20 mM]	2.36 mg [20 mM]
HCl	pH adjustor	adjusted to pH 6.0	adjusted to pH 6.0	adjusted to pH 6.0	adjusted to pH 6.0	adjusted to pH 6.0
NaOH	pH adjustor					

[0133]

For the prepared samples of Tables 6-1 and 6-2, the viscosity was evaluated by dynamic light scattering (DLS).

[0134]

[Dynamic light scattering (DLS)]

A standard curve of viscosity-apparent particle radius was prepared from apparent particle radii obtained by adding polystyrene particles to glycerin solutions of

50%, 60%, 65%, 70%, and 75%, using DynaPro Platereader (Wyatt). Next, an apparent radius was measured by adding polystyrene particles to a high concentration sample, and the viscosity was calculated from the standard curve of glycerin solutions.

[0135]

The range of viscosity is desired to be controlled to 1000 mPa·s or less, preferably 100 mPa·s or less, and more preferably 20 mPa·s or less.

[0136]

The evaluation results of viscosity obtained in this Example and Comparative Example are shown in Table 6-3 and Figure 5. For the samples added with arginine, an increase in the viscosity accompanying the increase in the concentration of the active ingredient was suppressed.

[0137]

On the other hand, the influence of pH on viscosity was not observed.

[0138]

[Table 6-3]

Sample No.	Active ingredient concentration	Buffer／pH	Additive	Viscosity（mPa·s)
F13	150 mg/mL	20 mM L-Histidine／pH 6	—	18.4
F14	120 mg/mL			11.4
F15	90 mg/mL			7.5
F1	150 mg/mL		140 mM Arginine-HCl	17.7
F2	120 mg/mL			8.7
F3	90 mg/mL			5.7
F16	150 mg/mL	20 mM Phosphoric acid／pH 6	—	28.4
F17	120 mg/mL			11.3
F18	90 mg/mL			6.3
F4	150 mg/mL		140 mM Arginine-HCl	16.2
F5	120 mg/mL			9.7
F6	90 mg/mL			5.0
F19	150 mg/mL	20 mM Citric acid／pH 5	—	22.8
F20	120 mg/mL			11.4
F21	90 mg/mL			5.3
F22	150 mg/mL	20 mM Citric acid／pH 6		22.2
F23	120 mg/mL			9.8
F24	90 mg/mL			5.7
F7	150 mg/mL		140 mM Arginine-HCl	18.1
F8	120 mg/mL			10.7
F9	90 mg/mL			5.7
F25	150 mg/mL	20 mM Succinic acid／pH 6	—	16.2
F26	120 mg/mL			8.2
F27	90 mg/mL			4.8
F10	150 mg/mL		140 mM Arginine-HCl	17.9
F11	120 mg/mL			11.1
F12	90 mg/mL			6.9

[0139]

<<Example 7: Stability evaluation>>

For a liquid preparation containing antibody A, the stability was evaluated. The formulation of the evaluation sample is shown in Table 7-1 below. The evaluation sample was prepared by, after cultivation and purification, carrying out buffer exchange to the formulation of Table 7-1, diluting the obtained protein drug solution with a solution containing the components other than antibody A described in Table 7-1, filtering it through a 0.22 μm filter, filling a glass vial with the filtrate, and carrying out capping and tightening.

[0140]

[Table 7-1]

Component	Function	G1 (/1mL)
Antibody A	Active ingredient	30 mg [approx. 0.21 mM]
$Na_2HPO_4 \cdot 12H_2O$	Buffer	0.82 mg [2 mM] (pH 5.7)
$NaH_2PO_4 \cdot 2H_2O$	Buffer	2.76 mg [18 mM] (pH 5.7)
L-Arginine· HCl	Stabilizer	29.49 mg [140 mM]
Polysorbate 80	Surfactant	0.20 mg [0.15 mM] (0.02 w/v%)

[0141]

In order to evaluate the stability of the liquid preparation, a storage stability test (at -20˚C for 12 months and at 5˚C for 12 months) of the sample was carried out. The quality of the antibody before and after the storage was evaluated by size exclusion chromatography (SEC), ion exchange chromatography (IEC), and hydrophobic interaction chromatography (HIC). The analysis conditions are as follows.

[0142]

[Size exclusion chromatography (SEC)]

A TSK guard column SWXL (Tosoh) and two G3000 SWXL columns for SEC (Tosoh) sequentially connected were connected to an HPLC system, and a mobile phase consisting of 20 mmol/L phosphoric acid and 1 mol/L NaCl pH 6.5 was flowed at a flow rate of 0.5 mL/min. Each sample was injected in an amount of 50 μg in terms of protein. The analysis time was 60 minutes, and the detection was carried out at UV 280 nm. The column temperature was set to 30˚C, and the sample temperature was set to 5˚C.

[0143]

[Ion exchange chromatography (IEC)]

A MabPac SCX10 column for IEC (Thermo) was connected to an HPLC system. A mobile phase consisting of 25 mmol/L MES pH 6.0 was connected to a mobile phase A line, and a mobile phase consisting of 25 mmol/L MES and 500 mmol/L NaCl pH 6.0 was connected to a mobile phase B line, and the mobile phases were flowed at a flow rate of 1 mL/min. The samples were diluted to 1 mg/mL with the mobile phase A, and 10 μL was injected. The analysis time was 70 minutes, and the IEC gradient program of Table 7-2 was applied. The detection was carried out at UV 280 nm. The column temperature was set to 35˚C, and the sample temperature was set to 5˚C.

[0144]

[Table 7-2]

Time (min.)	Mobile phase B %
0	15
5	15
50	30
50.1	100
60	100
60.1	15
70	15

[0145]

[Hydrophobic interaction chromatography (HIC)]

Two ProPac HIC-10 columns for HIC (Dionex) were connected to an HPLC system. A mobile phase consisting of 800 mmol/L ammonium sulfate and 20 mmol/L sodium phosphate pH7.0 was connected to a mobile phase A line, and a mobile phase consisting of 20 mmol/L sodium phosphate pH7.0 was connected to a mobile phase B line, and the mobile phases were flowed at a flow rate of 0.8 mL/min. The samples were diluted to 1 mg/mL with the mobile phase A, and 50 μL was injected. The HIC gradient program of Table 7-3 was applied. The detection was carried out at UV 280 nm. The column temperature was set to 30˚C, and the sample temperature was set to 25˚C.

[0146]

[Table 7-3]

Time (min.)	Mobile phase B %
0	0
2	0
47	20
49	20
54	100
56	0
66	0

[0147]

The areas of multimer peaks and degradant peaks detected by SEC, the area of the main peak detected by IEC, and the area of a hydrophilic peak detected by HIC were measured by an automatic analysis method to determine the amounts (%) thereof. The amounts are defined as percentage (%), by measuring the area of multimer peaks or degradant peaks detected by SEC by an automatic analysis method and dividing it by the sum of all peak areas including the main peak, by measuring the area of the main peak detected by IEC by an automatic analysis method and dividing it by the sum of all peak

areas other than the main peak, or by measuring the area of a hydrophilic peak detected by HIC by an automatic integration method and dividing it by the sum of all peak areas including the main peak. The main peak as used herein means the peak of the active body.

[0148]

The evaluation results of SEC, IEC, and HIC obtained in this Example are shown in Table 7-4. In this formulation, in both cases of the storage at -20˚C for 12 months and at 5˚C for 12 months, the quality in each index was within a suitable range, and it was confirmed that this formulation was stable.

[0149]

[Table 7-4]

Evaluation index	Storage conditions		
	Initial	-20°C 12 months	5°C 12 months
SEC Multimer %	0.68	0.94	1.81
SEC Degradant %	1.07	0.76	1.91
IEC Main peak %	69.36	67.94	58.76
HIC Hydrophilic peak %	11.24	11.70	26.49

INDUSTRIAL APPLICABILITY

[0150]

According to the present invention, a stable pharmaceutical composition comprising an anti-human TSLP receptor antibody, more particularly, a stable pharmaceutical composition comprising an anti-human TSLP receptor antibody, which suppresses the generation of chemically modified substances, such as deamidated forms and oxidized forms, or degradants or multimers, can be provided.

Although the present invention has been described with reference to specific embodiments, various changes and modifications obvious to those skilled in the art are possible without departing from the scope of the appended claims.

[FREE TEXT IN SEQUENCE LISTING]

[0151]

Features of "Artificial Sequence" are described in the numeric identifier <223> in the Sequence Listing. The nucleotide sequences of SEQ ID NOs: 2 and 4 in the Sequence Listing are ones of the H-chain and the L-chain of the anti-human TSLP Receptor antibody, respectively. The amino acid sequences of SEQ ID NOs: 1 and 3 are ones of the H-chain and the L-chain encoded by SEQ ID NOs: 2 and 4, respectively.

CLAIMS

1. A stable pharmaceutical composition comprising an anti-human TSLP receptor antibody, a pharmaceutically acceptable buffer of phosphoric acid in a concentration of 5 to 100 mmol/L; arginine or a pharmaceutically acceptable salt thereof, the concentration of the arginine or the pharmaceutically acceptable salt thereof being 700 mmol/L or less; and
a surfactant, being one, or two or more selected from the group consisting of polysorbates and poloxamer 188, whereby a content of the surfactant is 0.001 to 1% (w/v);

wherein the following (1) and/or (2) is contained as the anti-human TSLP receptor antibody:

(1) an anti-human TSLP receptor antibody comprising a heavy chain consisting of the amino acid sequence of SEQ ID NO: 1, and a light chain consisting of the amino acid sequence of SEQ ID NO: 3, and

(2) an anti-human TSLP receptor antibody consisting of amino acid sequences of an antibody generated by a post-translational modification of the anti-human TSLP receptor antibody of (1); and

wherein the post-translational modification includes pyroglutamylation at the N-terminus of a heavy chain variable region and/or lysine deletion at the C-terminus of a heavy chain.

2. The pharmaceutical composition according to claim 1, wherein the pharmaceutical composition is a liquid preparation, or a lyophilized preparation or a spray-dried preparation.

3. The pharmaceutical composition according to claim 2, wherein the pharmaceutical composition is a liquid preparation.

4. The pharmaceutical composition according to claim 2, wherein when the pharmaceutical composition is a liquid preparation, a pH of the liquid preparation is 5 to 6, or

when the pharmaceutical composition is a lyophilized preparation or a spray-dried preparation, a pH of a solution after reconstituting the preparation in water is 5 to 6.

5. The pharmaceutical composition according to claim 4, wherein the pharmaceutical composition is a liquid preparation, and the pH of the liquid preparation is 5 to 6.

6. The pharmaceutical composition according to any one of claims 1 to 5, wherein the surfactant is polysorbate 80.

7. The pharmaceutical composition according to any one of claims 1 to 6, wherein the content of the surfactant is 0.01 to 0.2% (w/v).

8. The pharmaceutical composition according to any one of claims 2 to 7, wherein when the pharmaceutical composition is a liquid preparation, a content of the anti-human TSLP receptor antibody is 0.007 to 2 mmol/L, or when the pharmaceutical composition is a lyophilized preparation or a spray-dried preparation, a content of a solution after reconstituting the preparation in water is 0.007 to 2 mmol/L.

9. The pharmaceutical composition according to any one of claims 1 to 8, when the pharmaceutical composition is stored, each content of a degradant and a multimer is 10% or less, or a content of a chemically modified substance is 50% or less.

10. The pharmaceutical composition according to claim 1, comprising an anti-human TSLP receptor antibody comprising a heavy chain consisting of the amino acid sequence of SEQ ID NO: 1, and a light chain consisting of the amino acid sequence of SEQ ID NO: 3.

11. The pharmaceutical composition according to claim 1, comprising an anti-human TSLP receptor antibody

consisting of amino acid sequences of an antibody generated by a post-translational modification of an anti-human TSLP receptor antibody comprising a heavy chain consisting of the amino acid sequence of SEQ ID NO: 1, and a light chain consisting of the amino acid sequence of SEQ ID NO: 3.

12. The pharmaceutical composition according to claim 1, comprising an anti-human TSLP receptor antibody comprising a heavy chain consisting of the amino acid sequence of amino acids 1-447 of SEQ ID NO: 1, and a light chain consisting of the amino acid sequence of SEQ ID NO: 3.

13. The pharmaceutical composition according to claim 1, comprising an anti-human TSLP receptor antibody comprising a heavy chain consisting of the amino acid sequence of SEQ ID NO: 1, and a light chain consisting of the amino acid sequence of SEQ ID NO: 3, and an anti-human TSLP receptor antibody comprising a heavy chain consisting of the amino acid sequence of amino acids 1-447 of SEQ ID NO: 1, and a light chain consisting of the amino acid sequence of SEQ ID NO: 3.

14. The pharmaceutical composition according to any one of claims 2 to 13, wherein formulation is liquid formulation comprising 0.2mmol/L(30mg/mL) anti-human TSLP receptor antibody, 20mmol/L phosphoric acid, 140 mmol/L L-Arginine Hydrochloride, and 0.02%(w/v) polysorbate 80.