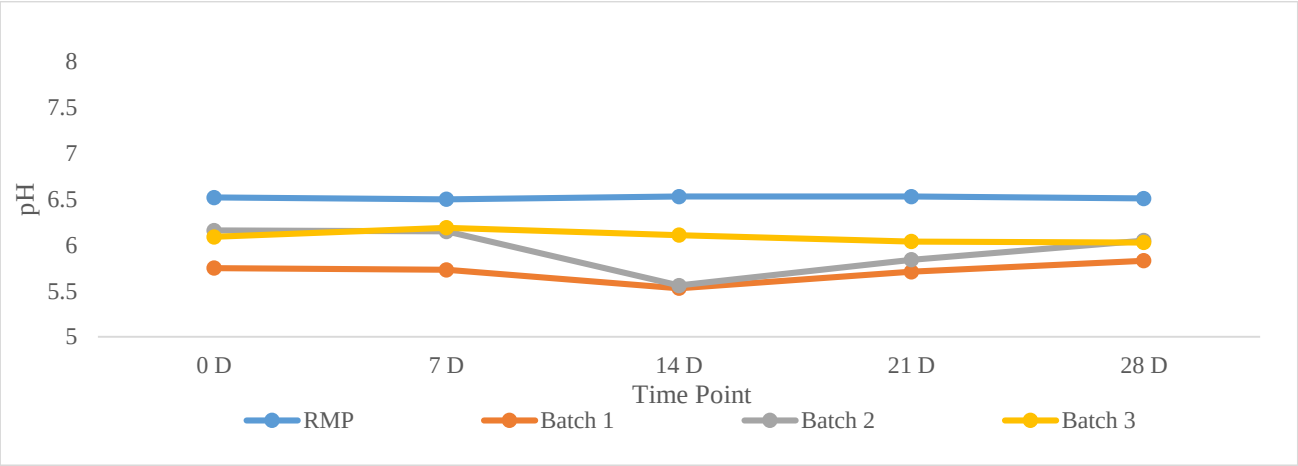

Figure - 1: pH trend analysis of RMP & Batch 1, 2, & 3 at 40˚ C

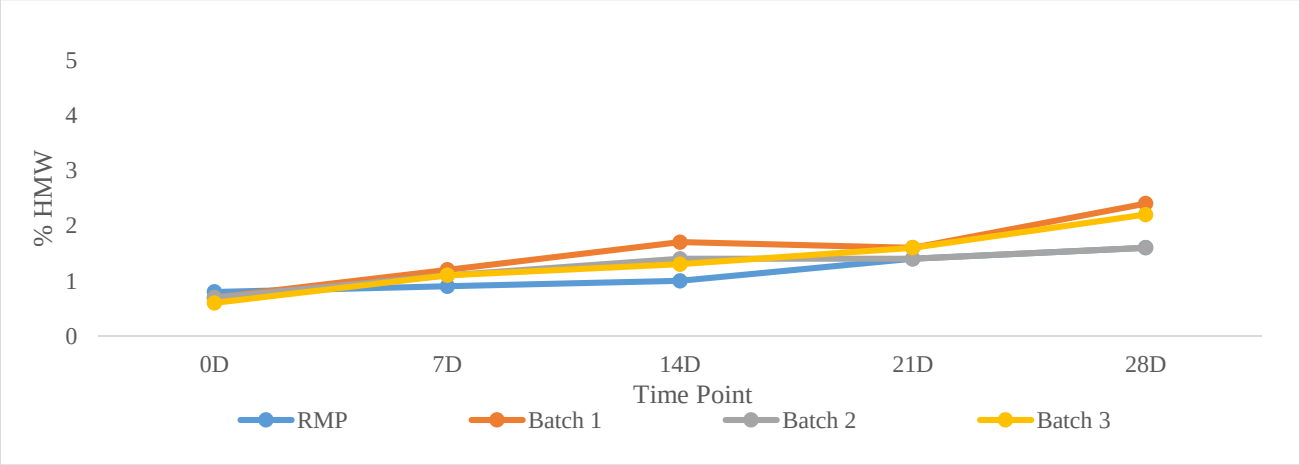

Figure - 2: Percentage HMW (SEC) trend analysis of RMP & Batch 1, 2, & 3 at 40 ºC

Dated: 27th June, 2023

Signature: _____

Name: Dr. Alpesh Pathak

Reg. No.: IN/PA-889

For and on behalf of the applicant

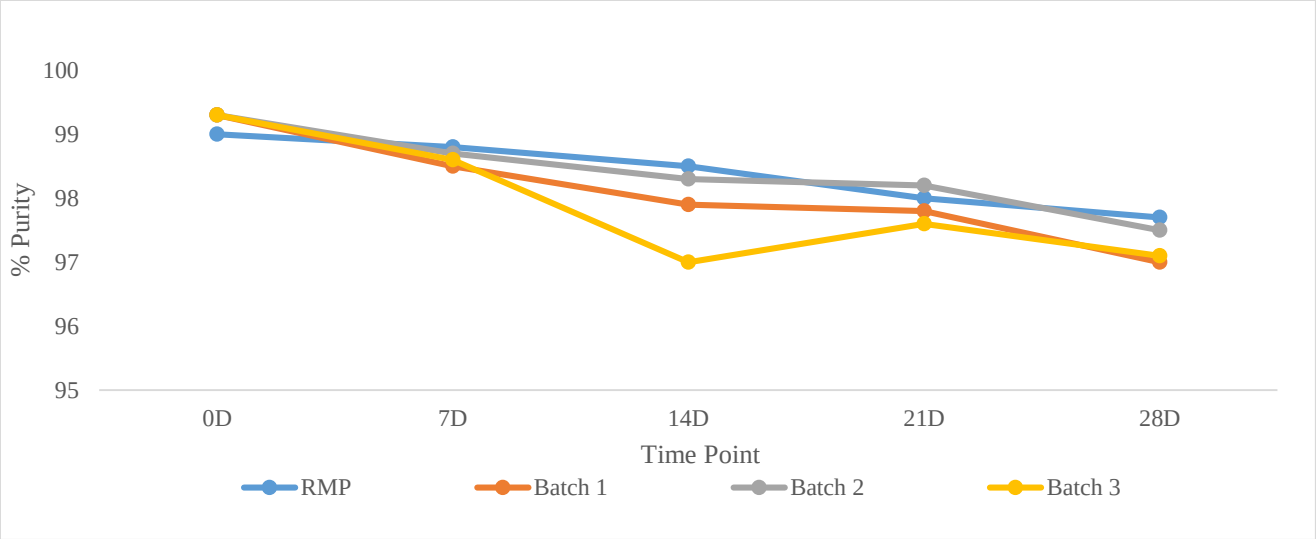

Figure - 3: Percentage Purity (SEC) trend analysis of RMP & Batch 1, 2, & 3 at 40ºC

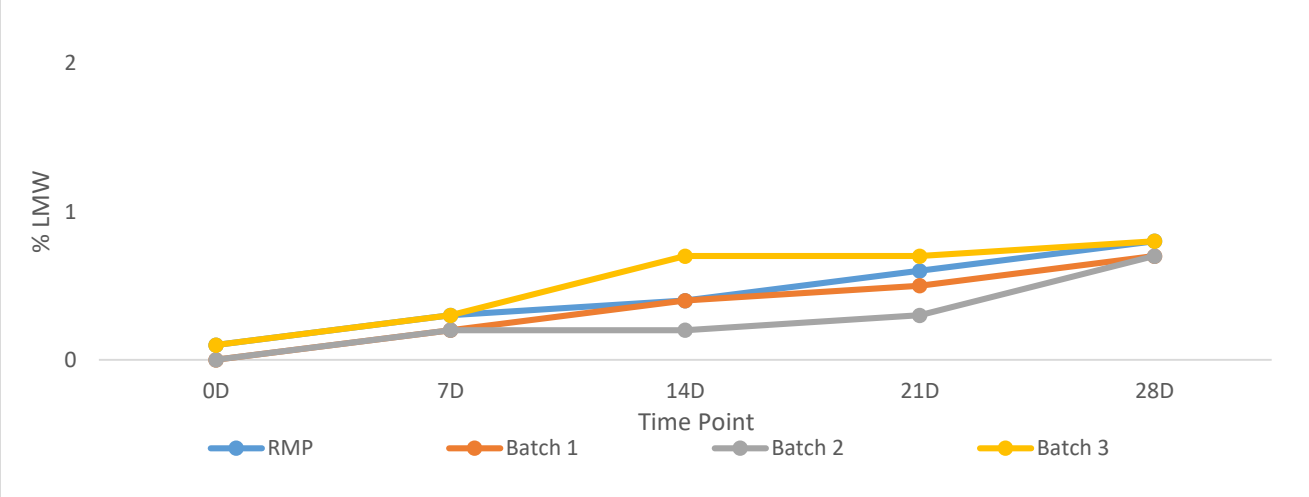

Figure - 4: Percentage LMW (SEC) trend analysis of RMP & Batch 1, 2, & 3 at 40º C

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Name: Dr. Alpesh Pathak

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For and on behalf of the applicant

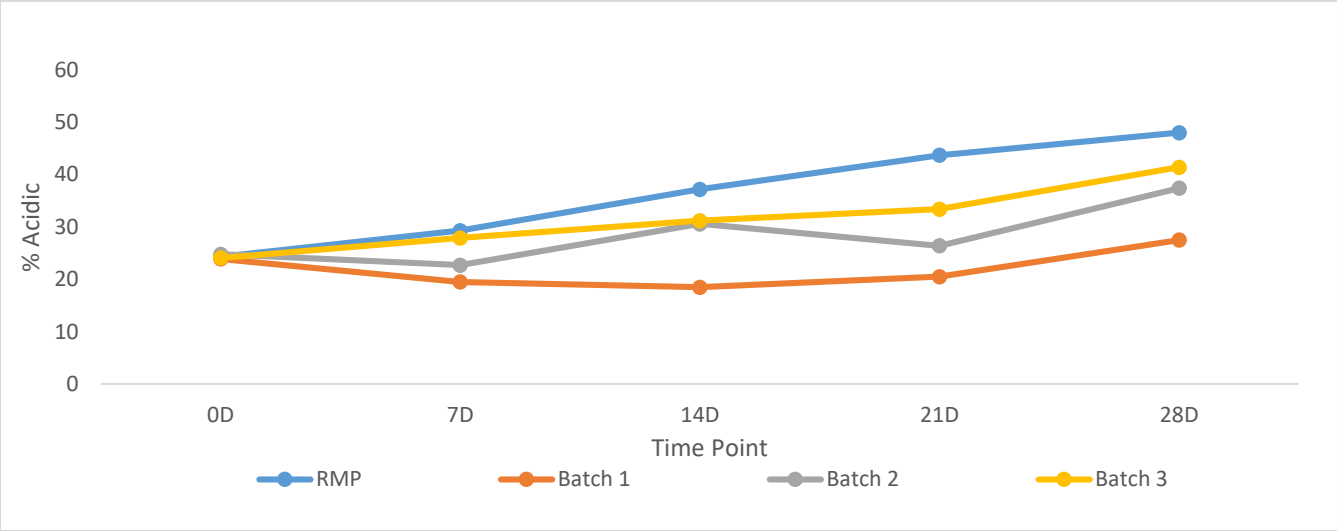

Figure - 5: Percentage Acidic (CEX) trend analysis of RMP & Batch 1, 2, & 3 at 40 ºC

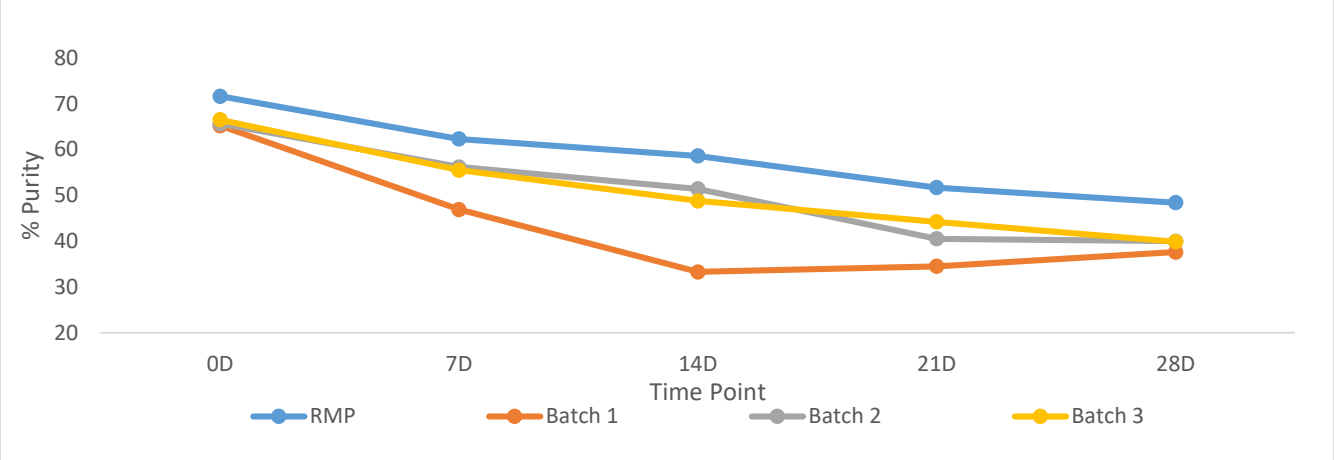

Figure - 6: Percentage Purity (CEX) trend analysis of RMP & Batch 1, 2, & 40 ºC

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Signature: _____

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For and on behalf of the applicant

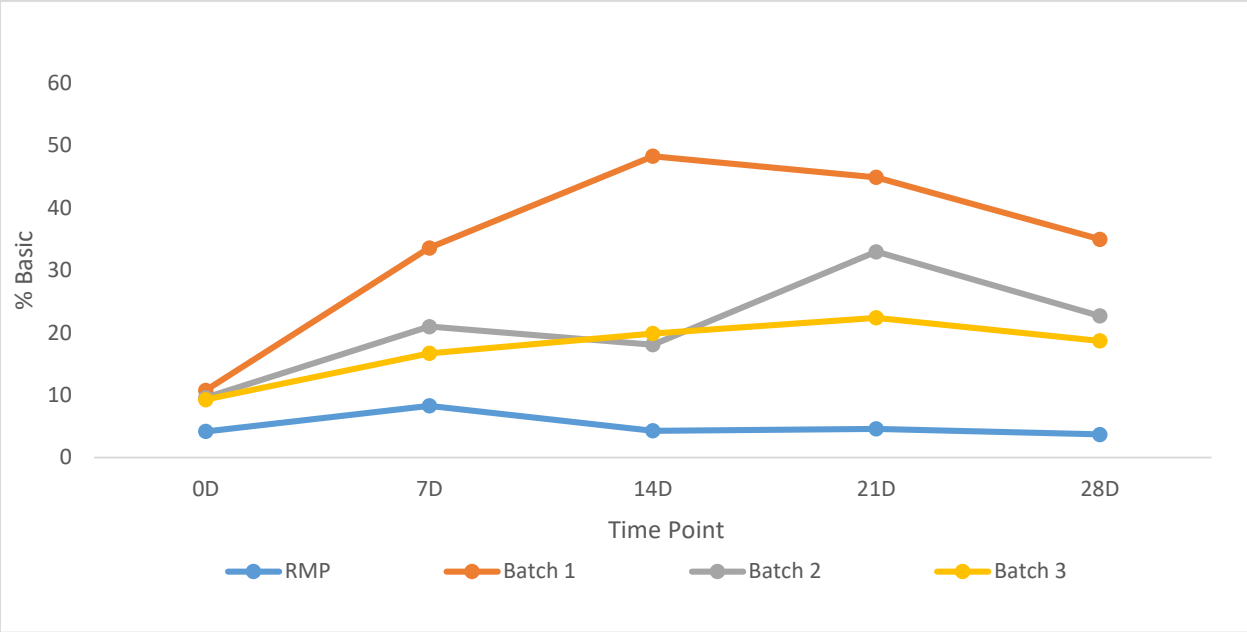


Figure - 7: Percentage Basic (CEX) trend analysis of RMP & Batch 1, 2, & 3 at 40 ºC

Dated: 27th June, 2023

Signature: _____

Name: Dr. Alpesh Pathak

Reg. No.: IN/PA-889

For and on behalf of the applicant

FORM 2

THE PATENTS ACT, 1970
(39 OF 1970)
&
The Patents Rules, 2003
COMPLETE SPECIFICATION
(See section 10; rule 13)

1. Title of the invention: **STABLE LIQUID FORMULATION OF AN ANTI-α4ß7 ANTIBODY**

2. Applicant(s)

(a) NAME: **INTAS PHARMACEUTICALS LIMITED**

(b) NATIONALITY: An Indian Company.

(c) ADDRESS: INTAS PHARMACEUTICALS LIMITED,
Corporate House, Nr. Sola Bridge, S. G. Highway, Thaltej,
Ahmedabad - 380054,
Gujarat, India

3. PREAMBLE TO THE DESCRIPTION

The following specification particularly describes the invention and the manner in which it is to be performed:

FIELD OF THE INVENTION

This application is related to Indian Provisional Application IN202221038828 filed 6th Jul, 2022 an is incorporated herein in its entirely.

FIELD OF THE INVENTION

The present invention relates to a stable liquid formulation of an anti-α4ß7 antibody.

BACKGROUND OF THE INVENTION

Advances in biotechnology have made it possible to produce a variety of proteins for pharmaceutical applications using recombinant DNA techniques. Because proteins are larger and more complex than traditional organic and inorganic drugs (i.e., possessing multiple functional groups in addition to complex three- dimensional structures), the formulation of such proteins poses special problems. For a protein to remain biologically active, a formulation must preserve the conformational integrity of at least a core sequence of the protein's amino acids, while at the same time protecting the protein's multiple functional groups from degradation. Proteins may suffer from a lack of stability, and monoclonal and polyclonal antibodies in particular may be relatively unstable. Numerous characteristics may affect a protein's stability. In fact, even in the case of purified antibodies, the antibody structures may be heterogenous, which further complicates the formulation of such systems. Moreover, the excipients included in antibody formulations preferably minimize any potential immune response.

In the case of antibodies, preservation of the conformational integrity is even more important. Degradation pathways for proteins can involve chemical instability (i.e., any process which involves modification of the protein by bond formation or cleavage resulting in a new chemical entity) or physical instability (i.e., changes in the higher order structure of the protein). Chemical instability is manifested in, for example, deamidation, isomerization, hydrolysis, oxidation, fragmentation, glycan beta elimination or disulfide exchange. Physical instability can result from denaturation, aggregation, precipitation or adsorption, for example. The four most common protein degradation pathways are protein fragmentation, aggregation, deamidation, and oxidation. Consequences of chemical or physical instability of therapeutic protein include a lowering of the effective administered dose, decreased safety of the therapy due to, for example irritation or immunological reactivity, and more frequent manufacturing due to short shelf life.

Over the past two decades, recombinant DNA technology has led to the commercialization of many proteins, particularly antibody therapeutics. The effectiveness of these therapeutic antibodies is majorly dependent on the stability, route of administration and their dosage forms and concentrations. This in turn, necessitates therapeutic antibodies to be formulated appropriately to retain the stability and activity of a therapeutic antibody.

Formulations for each route of administration and dosage forms may be unique and, therefore, have specific requirements. Solid dosage forms, such as lyophilized powders, are generally more stable than liquid (aqueous) formulations. However, reconstitution of the lyophilized formulation requires a significant vial overfill, care in handling and involves high production cost relative to a liquid formulation. While liquid formulations are advantageous in these and are usually preferred for injectable protein therapeutics (in terms of convenience for the end user and ease of preparation for the manufacturer), this form may not always be feasible given the susceptibility of proteins to denaturation, aggregation and oxidation under stresses such as temperature, pH changes, agitation etc.,. All of these stress factors could result in the loss of biological activity of a therapeutic protein / antibody. In particular, high concentration liquid formulations are susceptible to degradation and/or aggregation. Nevertheless, high concentration formulations may be desirable for subcutaneous or intravenous route of administration, as the frequency of administration and injection volume is reduced. On the other hand, specific treatment schedule and dosing might require a low concentration formulation and prefer intravenous route of administration for more predictable delivery and complete bioavailability of the therapeutic drug.

Hence, designing a formulation that is stable at high or low concentrations of the therapeutic protein /antibody, aiding in different route of administration (intravenous or subcutaneous), pose a significant developmental challenge. Further, every protein or antibody with its unique characteristics and properties of degradation, adds to the complexity in the development of a stable formulation and may demand a specific formulation.

WO2012151247 discloses stable liquid pharmaceutical formulation of Vedolizumab comprising Citrate or EDTA as chelating agent or antioxidant. It further discloses free amino acid which is selected from the group consisting of histidine, alanine, arginine, glycine, glutamic acid and combinations thereof. More particularly, it discloses stable liquid pharmaceutical formulation comprising a mixture of an anti-α4β7 antibody, citrate, histidine, arginine, and polysorbate 80.

A formulation combination with increased concentration of protein and /or stabilizers may increase the viscosity of the formulation, in turn increasing the injection time and pain at the site of injection and also pose difficulties during processing of the drug substance. Hence, it is necessary to develop an improved formulation, which contains minimal number or concentration of excipients, yet stabilizing the drug at a wide range of its concentration.

OBJECTS OF THE INVENTION

The main object of the present invention is to provide stable liquid pharmaceutical formulation comprising a mixture of an anti-α4ß7 antibody, a buffer, stabilizer, antioxidant, viscosity reducer and surfactant.

Another object of the present invention is to provide stable liquid pharmaceutical formulation comprising anti-α4ß7 antibody, a buffer, stabilizer, L-methionine as an antioxidant, L-lysine HCl as a viscosity reducer and surfactant.

Another object of the present invention is provide stable liquid pharmaceutical formulation of an anti-α4ß7 antibody comprising buffer, sugar, viscosity reducer, antioxidant and surfactant at pH about 5.8 to 6.8, wherein the molar ratio of sugar to anti-α4β7 antibody (mole:mole) is less than 150:1.

Another object of the present invention is to provide stable liquid pharmaceutical formulation comprising at least about 50 mg/ml to about 220 mg/ml anti-α4ß7 antibody, a buffering agent, stabilizer, L-methionine as an antioxidant, L-lysine HCl as a viscosity reducer and surfactant.

Another object of the present invention is to provide stable liquid pharmaceutical formulation of an anti-α4ß7 antibody comprising a buffer, stabilizer, viscosity reducer, antioxidant and surfactant, wherein the formulation has pH about 5.8 to 6.8.

Another object of the present invention is provide stable liquid pharmaceutical formulation of an anti-α4ß7 antibody comprising sucrose as a stabilizer, L-lysine HCl as a viscosity reducer and L-methionine as an antioxidant, wherein the formulation has pH about 5.8 to 6.8..

Another object of the present invention is provide stable liquid pharmaceutical formulation of an anti-α4ß7 antibody comprising L-histidine, L-histidine monohydrochloride, sucrose, L-lysine HCl, L-methionine and polysorbate 80, wherein the formulation has pH about 5.8 to 6.8.

Another object of the present invention is to provide stable liquid pharmaceutical formulation of an anti-α4ß7 antibody comprising buffer, stabilizer, viscosity reducer, antioxidant and surfactant; wherein the anti-α4ß7 antibody is at a concentration of about 50-220 mg/mL, buffer is at a concentration range of 0-100 mM, stabilizer is at a concentration range of 0-100 mg/mL, viscosity reducer is at a concentration range of 0-10 mg/mL, antioxidant is at a concentration range of 0-2 mg/mL and surfactant is at a concentration range of 0-2 mg/mL, wherein the formulation has pH about 5.8 to 6.8.

Another object of the present invention is to provide stable liquid pharmaceutical formulation of an anti-α4ß7 antibody comprising sucrose as a stabilizer, L-lysine HCl as a viscosity reducer and L-methionine as an antioxidant; wherein the anti-α4ß7 antibody is at concentration of about 50-220 mg/mL, sucrose is in the concentration range of 0-100 mg/mL, L-lysine HCl is in the concentration range of 0-10 mg/mL and L-methionine is in the concentration range of 0-2 mg/mL, wherein the formulation has pH about 5.8 to 6.8.

Another object of the present invention is to provide stable liquid pharmaceutical formulation of an anti-α4ß7 antibody comprising 0-100 mM histidine/histidine monohydrochloride, 0-100 mg/mL sucrose, 0-10 mg/mL L-lysine HCl, 0-2 mg/mL L-methionine and 0-2 mg/mL polysorbate 80, wherein the formulation has pH about 5.8 to 6.8.

Another object of the present invention is to provide stable liquid pharmaceutical formulation of an anti-α4ß7 antibody comprising 50 mM histidine/histidine HCl, 45 mg/mL sucrose, 8 mg/mL L-lysine HCl, 1.49 mg/mL L-methionine and 2 mg/mL polysorbate 80, wherein the formulation has pH about 5.8 to 6.8.

SUMMARY OF THE INVENTION

The main aspect of the present invention is to provide stable liquid pharmaceutical formulation comprising a mixture of an anti-α4ß7 antibody, a buffer, stabilizer, antioxidant, viscosity reducer and surfactant.

Another aspect of the present invention is to provide stable liquid pharmaceutical formulation comprising anti-α4ß7 antibody, a buffer, stabilizer, L-methionine as an antioxidant, L-lysine HCl as a viscosity reducer and surfactant.

Another aspect of the present invention is provide stable liquid pharmaceutical formulation of an anti-α4ß7 antibody comprising buffer, sugar, viscosity reducer, antioxidant and surfactant at pH about 5.8 to 6.8, wherein the molar ratio of sugar to anti-α4β7 antibody (mole:mole) is less than 150:1.

Another aspect of the present invention is to provide stable liquid pharmaceutical formulation comprising at least about 50 mg/ml to about 220 mg/ml anti-α4ß7 antibody, a buffering agent, stabilizer, L-methionine as an antioxidant, L-lysine HCl as a viscosity reducer and surfactant.

Another aspect of the present invention is to provide stable liquid pharmaceutical formulation of an anti-α4ß7 antibody comprising a buffer, stabilizer, viscosity reducer, antioxidant and surfactant, wherein the formulation has pH about 5.8 to 6.8.

Another aspect of the present invention is provide stable liquid pharmaceutical formulation of an anti-α4ß7 antibody comprising sucrose as a stabilizer, L-lysine HCl as a viscosity reducer and L-methionine as an antioxidant, wherein the formulation has pH about 5.8 to 6.8.

Another aspect of the present invention is provide stable liquid pharmaceutical formulation of an anti-α4ß7 antibody comprising L-histidine, L-histidine monohydrochloride, sucrose, L-lysine HCl, L-methionine and polysorbate 80, wherein the formulation has pH about 5.8 to 6.8.

Another aspect of the present invention is to provide stable liquid pharmaceutical formulation of an anti-α4ß7 antibody comprising buffer, stabilizer, viscosity reducer, antioxidant and surfactant; wherein the anti-α4ß7 antibody is at a concentration of about 50-220 mg/mL, buffer is at a concentration range of 0-100 mM, stabilizer is at a concentration range of 0-100 mg/mL, viscosity

reducer is at a concentration range of 0-10 mg/mL, antioxidant is at a concentration range of 0-2 mg/mL and surfactant is at a concentration range of 0-2 mg/mL, wherein the formulation has pH about 5.8 to 6.8.

Another aspect of the present invention is to provide stable liquid pharmaceutical formulation of an anti-α4ß7 antibody comprising sucrose as a stabilizer, L-lysine HCl as a viscosity reducer and L-methionine as an antioxidant; wherein the anti-α4ß7 antibody is at concentration of about 50-220 mg/mL, sucrose is in the concentration range of 0-100 mg/mL, L-lysine HCl is in the concentration range of 0-10 mg/mL and L-methionine is in the concentration range of 0-2 mg/mL, wherein the formulation has pH about 5.8 to 6.8.

Another aspect of the present invention is to provide stable liquid pharmaceutical formulation of an anti-α4ß7 antibody comprising 0-100 mM histidine/histidine monohydrochloride, 0-100 mg/mL sucrose, 0-10 mg/mL L-lysine HCl, 0-2 mg/mL L-methionine and 0-2 mg/mL polysorbate 80, wherein the formulation has pH about 5.8 to 6.8.

Another aspect of the present invention is to provide stable liquid pharmaceutical formulation of an anti-α4ß7 antibody comprising 50 mM histidine/histidine HCl, 45 mg/mL sucrose, 8 mg/mL L-lysine HCl, 1.49 mg/mL L-methionine and 2 mg/mL polysorbate 80, wherein the formulation has pH about 5.8 to 6.8.

BRIEF DESCRIPTION OF DRAWING

In order that disclosure may be readily understood and put into practical effect, reference will now be made to exemplary embodiments as illustrated with reference to the accompanying figures. The figures with a detailed description below, are incorporated in and form part of the specification, and serve to further illustrate the embodiments and explains various principles and advantages, in accordance with the present disclosure wherein:

FIGURE 1: pH trend analysis of RMP & batch 1, 2 & 3 at 40˚ C.

FIGURE 2: Percentage HMW (SEC) trend analysis of RMP & Batch 1, 2 & 3 at 40˚ C.

FIGURE 3: Percentage purity (SEC) trend analysis of RMP & Batch 1, 2 & 3 at 40˚ C.

FIGURE 4: Percentage LMW (SEC) trend analysis of RMP & Batch 1, 2 & 3 at 40˚ C.

FIGURE 5: Percentage Acidic (CEX) trend analysis of RMP & Batch 1, 2 & 3 at 40˚ C.

FIGURE 6: Percentage Purity (CEX) trend analysis of RMP & Batch 1, 2 & 3 at 40˚ C.

FIGURE 7: Percentage Basic (CEX) trend analysis of RMP & Batch 1, 2 & 3 at 40˚ C.

DETAILED DESCRIPTION OF THE INVENTION

DEFINITION

The following definitions are provided to facilitate understanding of certain terms used throughout the specification.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by those of ordinary skill in the art to which the invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of particular embodiments, preferred embodiments of compositions, methods and materials are described herein. For the purposes of the present disclosure, the following terms are defined below.

The articles "a," "an," and "the" are used herein to refer to one or to more than one (i.e., to at least one, or to one or more) of the grammatical object of the article. By way of example, "an element" means one element or one or more elements.

The words "comprise", "comprises", and "comprising" are to be interpreted inclusively rather than exclusively. The words "consist", "consisting", and its variants, are to be interpreted exclusively, rather than inclusively. While various embodiments in the specification are presented using “comprising” language, under other circumstances, a related embodiment is also intended to be interpreted and described using “consisting of” or “consisting essentially of” language.

The term "pharmaceutical formulation" refers to a preparation that contains an anti- α4ß7 antibody in such form as to permit the biological activity of the antibody to be effective, and which contains

no additional components which are unacceptably toxic to a subject to which the formulation would be administered.

A "stable" formulation is one in which the antibody therein substantially retains its physical stability and/or chemical stability and/or its biological activity upon storage. In one aspect, the formulation substantially retains its physical and chemical stability, as well as its biological activity upon storage. The storage period is generally selected based on the intended shelf-life of the formulation. Various analytical techniques for measuring protein stability are available in the art.

Stability studies provides evidence of the quality of an antibody under the influence of various environmental factors during the course of time. ICH's"Q1A: Stability Testing of New Drug Substances and Products," states that data from accelerated stability studies can be used to evaluate the effect of short-term excursions higher or lower than label storage conditions that may occur during the shipping of the antibodies.

Various analytical methods are available for measuring the physical and chemical degradation of the antibody in the pharmaceutical formulations. An antibody "retains its physical stability" in a pharmaceutical formulation if it shows substantially no signs of aggregation, precipitation and/or denaturation upon visual examination of color and/or clarity, or as measured by UV light scattering or by size exclusion chromatography. An antibody is said to "retain its chemical stability" in a pharmaceutical formulation when its shows no or minimal formation of product variants which may include variants as a result of chemical modification of antibody of interest such as deamination, oxidation etc. Analytical methods such as ion exchange chromatography and hydrophobic ion chromatography may be used to investigate the chemical product variants.

As used herein, "buffering agent" refers to a buffer that resists changes in pH by the action of its acid-base conjugate components. The buffering agent may be present in a liquid or solid formulation of the invention. In some embodiments, the buffering agent of this invention adjusts the pH of the formulation to about 5.8 to 6.8. In one aspect, examples of buffering agents that alone or in combination, will control the pH in the 5.8 to 6.8 range include acetate, succinate, gluconate, histidine, citrate, phosphate, maleate, cacodylate, 2-[N- morpholinojethanesulfonic acid (MES), bis(2- hydroxyethyl)iminotris[hydroxymethyl]methane (Bis-Tris), N-[2-acetamido]-2-

iminodiacetic acid (ADA), glycylglycine and other organic acid buffers. In another aspect, the buffering agent herein is histidine.

A "histidine buffer" is a buffer comprising histidine ions. Examples of histidine buffers include histidine chloride, histidine acetate, histidine phosphate, histidine sulfate solutions. The histidine buffer or histidine-HCl buffer has a between about pH 5.8 to 6.8.

A "surfactant" herein refers to an agent that lowers surface tension of a liquid. The surfactant can be a nonionic surfactant. In one aspect, examples of surfactants herein include polysorbate (polyoxyethylene sorbitan monolaurate, for example, polysorbate 20 and, polysorbate 80).

An "antioxidant" herein refers to an agent that inhibits the oxidation of other molecules. Examples of antioxidants herein include L-methionine, lipoic acid, uric acid, glutathione, tocopherol, carotene, lycopene, cysteine, phosphonate compounds, e.g., etidronic acid, desferoxamine and malate.

An ‘anti-α4ß7 antibody’ herein refers to Vedolizumab antibody.

The main embodiment of the present invention is to provide stable liquid pharmaceutical formulation comprising a mixture of an anti-α4ß7 antibody, a buffer, stabilizer, antioxidant, viscosity reducer and surfactant.

Another embodiment of the present invention is to provide stable liquid pharmaceutical formulation comprising anti-α4ß7 antibody, a buffer, stabilizer, L-methionine as an antioxidant, L-lysine HCl as a viscosity reducer and surfactant.

Another embodiment of the present invention is provide stable liquid pharmaceutical formulation of an anti-α4ß7 antibody comprising buffer, sugar, viscosity reducer, antioxidant and surfactant at pH about 5.8 to 6.8, wherein the molar ratio of sugar to anti-α4β7 antibody (mole:mole) is less than 150:1.

In another embodiment of the present invention sugar is sucrose.

Another embodiment of the present invention is to provide stable liquid pharmaceutical formulation comprising at least about 50 mg/ml to about 220 mg/ml anti-α4ß7 antibody, a

buffering agent, stabilizer, L-methionine as an antioxidant, L-lysine HCl as a viscosity reducer and surfactant.

In another embodiment of the present invention, the stable liquid pharmaceutical formulation of anti-α4ß7 antibody is high concentration formulation.

In another embodiment of the present invention, the concentration of anti-α4ß7 antibody in the formulation is about 160 mg/ml.

Another embodiment of the present invention is to provide stable liquid pharmaceutical formulation of an anti-α4ß7 antibody comprising a buffer, stabilizer, viscosity reducer, antioxidant and surfactant, wherein the formulation has pH about 5.8 to 6.8.

Another embodiment of the present invention is provide stable liquid pharmaceutical formulation of an anti-α4ß7 antibody comprising sucrose as a stabilizer, L-lysine HCl as a viscosity reducer and L-methionine as an antioxidant, wherein the formulation has pH about 5.8 to 6.8.

Another embodiment of the present invention is provide stable liquid pharmaceutical formulation of an anti-α4ß7 antibody comprising L-histidine, L-histidine monohydrochloride, sucrose, L-lysine HCl, L-methionine and polysorbate 80, wherein the formulation has pH about 5.8 to 6.8.

Another embodiment of the present invention is to provide stable liquid pharmaceutical formulation of an anti-α4ß7 antibody comprising buffer, stabilizer, viscosity reducer, antioxidant and surfactant; wherein the anti-α4ß7 antibody is at a concentration of about 50-220 mg/mL, buffer is at a concentration range of 0-100 mM, stabilizer is at a concentration range of 0-100 mg/mL, viscosity reducer is at a concentration range of 0-10 mg/mL, antioxidant is at a concentration range of 0-2 mg/mL and surfactant is at a concentration range of 0-2 mg/mL, wherein the formulation has pH about 5.8 to 6.8.

Another embodiment of the present invention is to provide stable liquid pharmaceutical formulation of an anti-α4ß7 antibody comprising sucrose as a stabilizer, L-lysine HCl as a viscosity reducer and L-methionine as an antioxidant; wherein the anti-α4ß7 antibody is at concentration of about 50-220 mg/mL, sucrose is in the concentration range of 0-100 mg/mL, L-

lysine HCl is in the concentration range of 0-10 mg/mL and methionine is in the concentration range of 0-2 mg/mL, wherein the formulation has pH about 5.8 to 6.8.

Another embodiment of the present invention is to provide stable liquid pharmaceutical formulation of an anti-α4ß7 antibody comprising 0-100 mM histidine/histidine monohydrochloride, 0-100 mg/mL sucrose, 0-10 mg/mL L-lysine HCl, 0-2 mg/mL L-methionine and 0-2 mg/mL polysorbate 80, wherein the formulation has pH about 5.8 to 6.8.

Another embodiment of the present invention is to provide stable liquid pharmaceutical formulation of an anti-α4ß7 antibody comprising 50 mM histidine/histidine HCl, 45 mg/mL sucrose, 8 mg/mL L-lysine HCl, 1.49 mg/mL L-methionine and 2 mg/mL polysorbate 80, wherein the formulation has pH about 5.8 to 6.8.

In another embodiment of the present invention the concentration of polysorbate 80 in the formulation of anti-α4ß7 antibody is 0.2% w/v.

In another embodiment of the present invention the concentration of anti-α4ß7 antibody in the formulation is about 160 mg/ml.

In another embodiment of the present invention formulation of anti-α4ß7 antibody can be administered by subcutaneous route or intravenous route.

The embodiments of the present invention are further described using specific examples herein after. The examples are provided for better understanding of certain embodiments of the invention and not, in any manner, to limit the scope thereof. Possible modifications and equivalents apparent to those skilled in the art using the teachings of the present description and the general art in the field of the invention shall also from the part of this specification and are intended to be included within the scope of it.

EXAMPLES:

EXAMPLE 1: SCREENING OF FORMULATION EXCIPIENTS

An anti-α4ß7 antibody, Vedolizumab, suitable for storage in the present pharmaceutical composition was produced by standard methods known in the art. For example, Vedolizumab which was prepared by recombinant expression of immunoglobulin light and heavy chain genes in a mammalian host cell such as Chinese Hamster Ovary cells. Further, the expressed Vedolizumab was harvested and the crude harvest was subjected to standard downstream process steps that include purification, filtration and optionally dilution or concentration steps. For example, the crude harvest of Vedolizumab may be purified using standard chromatography techniques such as affinity chromatography, ion-exchange chromatography and combinations thereof. The purified Vedolizumab solution can additionally be subjected to one or more filtration steps, and the solution obtained was subjected to further formulation studies.

Method of preparation: Vedolizumab formulation was prepared in composition given in the table 1 by dissolving the excipients in water for injection. The protein concentration was set to 108 mg/0.68 mL (PFS)/ 158.8 mg/ mL and the pH of the formulation is set to 5.8 to 6.8.

0.68 mL of drug product solution was filled in 1 mL Syringe barrel-USP type I siliconized and stopper them with plunger stopper and store the filled PFS in between 2˚ C and 8˚ C.

Different excipients with different concentration were tested. Total 22 formulations were prepared with different concentration of excipients as shown in below table 1.

Table 1: Composition of Different Formulations

Components	**Active Protein**	**Buffer (mg/mL)**		**Antioxidant (mg/mL)**	**Stabilizer, viscosity and Isotonicity modifier**								**Nonionic surfactant (mg/mL)**		**pH**
Ingredient	Vedolizumab DS (mg/mL)	L-Histidine	L-Histidine monohydrochloride	L-Methionine	NaCl	L-Lysine HCl	Sucrose	Mannitol	Sorbitol	α,α trehalose dihydrate	L-Proline	L-Arginine HCl	Polysorbate 80	Polysorbate 20	
F-10	158.8	4.59	4.28	1.49	8.8	--	--	--	--	--	--	--	2	--	6.3 - 6.6
F-11					--	8.0	--	--	--	--	--	--	2	--	6.6
F-19					6.0	--	--	--	--	--	--	--	2	--	6.6
F-20					--	8.0	45	--	--	--	--	--	2	--	5.8 - 6.8
F-21					--	6.0	45	--	--	--	--	--	2	--	6.6
F-22					6.0	6.0	--	--	--	--	--	--	2	--	6.6
F-24					6.0	6.0	25	--	--	--	--	--	2	--	6.6
F-25					6.0	6.0	--	20	--	--	--	--	2	--	6.6
F-26					6.0	6.0	--	--	20	--	--	--	2	--	6.6
F-27					6.0	6.0	--	--	--	40	--	--	2	--	6.6
F-28					--	8.0	--	33	--	--	--	--	2	--	6.3 - 6.6
F-29					6.0	--	--	25	--	--	--	--	2	--	6.6
F-30					--	--	30	--	--	--	25	--	2	--	6.6
F-31					--	--	--	15	--	--	25	--	2	--	6.6
F-32					4.0	8.0	25	--	--	--	--	--	2	--	6.6
F-33					4.0	4.0	35	--	--	--	--	--	2	--	6.6
F-34					--	4.0	--	40	--	--	--	--	2	--	6.6
F-35					--	--	--	--	--	--	--	26.34	2	--	6.6
F-36					--	--	--	--	--	--	--	26.34	--	1	6.6
F-37					--	8.0	50	--	--	--	--	--	2	--	6.6
F-38					--	--	15	--	--	--	--	26.34	2	--	6.6
F-39					--	--	--	--	--	--	--	42.00	2	--	6.3 - 6.6

The Vedolizumab SC formulation drug product was formulated in above mention different buffers and putted on stress stability (40 ˚C ± 2 ˚C and 50 ˚C) and evaluated for analytical techniques as below table 2 and table 3:

Table 2: Stability Study Plan at 40 ˚C ± 2 ˚C

Analytical Test	Time Point (Days) for DP		
	0 D	**14 D**	**28 D**
Physical appearance	X	X	X
Osmolality	X	NA	NA
pH	X	X	X
Protein concentration	X	X	X
CEX-HPLC	X	X	X
SEC-HPLC	X	X	X
Pro-A oxidation	X	X	X
Relative Potency	X	NA	X
CE-SDS (NR)	X	NA	X
X: Testing time point			
NA: Not Applicable			

Table 3: Stability Study Plan at 50˚C

Analytical Test	Time Point (Days) for DP		
	0 D	**2 D/ 3 D**	**7 D**
Physical appearance	X	NA	NA
pH	X	NA	NA
Protein concentration	X	X	X
CEX-HPLC	X	X	X
SEC-HPLC	X	X	X
Pro-A oxidation	X	X	X
Relative Potency	X	NA	NA
CE-SDS (NR)	X	NA	X
X: Testing time point			
NA: Not Applicable			

EXAMPLE2: STRESS STABILITY STUDY

A) Physical appearance:

All the samples were observed to be colorless to yellow solution

B) pH:

Table 4: pH of RMP & Formulation (F-10 to F-39) at 50˚ C

Buffers	pH		
	0 D	**2 D**	**7 D**
RMP	6.56	NA	6.54
F-10	6.75	6.78	6.77
F-11	6.34	6.36	6.32
F-19	6.76	6.77	6.75
F-20	6.69	6.69	6.69
F-21	6.75	6.76	6.75
F-22	6.77	6.73	6.77
F-24	6.76	6.82	6.81
F-25	6.82	6.85	6.84
F-26	6.83	6.85	6.84
F-27	6.84	6.84	6.84
F-28	6.71	6.83	6.82
F-29	6.85	6.85	6.84
F-30	6.83	6.80	6.79
F-31	6.80	6.78	6.79
F-32	6.52	6.52	6.52
F-33	6.53	6.54	6.53
F-34	6.47	NA	6.45
F-35	6.66	6.68	6.63
F-36	6.67	6.68	6.64
F-37	6.67	6.69	6.66
F-38	6.67	6.67	6.64
F-39	6.36	6.39	6.39

Observation: Based on 7 days stress data the pH of the most of formulation were comparable with the reference formulation (RMP).

C) Protein Concentration:

Table 5: Protein Concentration of RMP & Formulation (F-10 to F-39) at 50˚ C

Buffers	Protein concentration		
	0 D	**2 D**	**7 D**
RMP	154.92	NA	152.94
F-10	161.46	165.10	163.81
F-11	143.49	142.71	143.35
F-19	159.41	162.55	160.07
F-20	160.80	169.42	172.75
F-21	164.20	163.18	162.27
F-22	163.40	164.27	162.45
F-24	152.74	152.84	155.60
F-25	156.70	153.42	158.63
F-26	156.33	157.25	158.21
F-27	157.35	158.25	160.99
F-28	157.17	154.65	162.45
F-29	159.36	158.79	159.07
F-30	160.01	155.47	160.94
F-31	162.22	162.54	164.53
F-32	157.41	157.81	158.71
F-33	157.85	156.59	165.02
F-34	161.99	NA	169.51
F-35	161.82	166.60	171.56
F-36	161.78	174.97	169.68
F-37	159.96	175.55	176.20
F-38	NA	NA	NA
F-39	NA	NA	NA

NA: Not analyzed

Observation: Based on 7 days stress data the Protein concentration of most of formulations were comparable with the reference formulation (RMP)

D) CEX- HPLC

Table 6: CEX data (% Acidic, % Purity and % Basic) of RMP & Formulation (F-10 to F-39) at 50˚ C

Buffers	Percentage Acidic			Percentage Purity			Percentage Basic		
	0 D	3 D	7 D	0 D	3 D	7 D	0 D	3 D	7 D
RMP	24.8	NA	43.1	70.9	NA	47.1	4.5	NA	9.7
F-10	29.1	33.3	43.6	57.7	51.0	43.9	13.2	15.6	12.4
F-11	NA	NA	NA	NA	NA	NA	NA	NA	NA
F-19	28.8	32	44.8	57.6	52.6	42.3	13.5	15.3	12.9
F-20	26.8	32.5	41.7	66.9	56.2	47.9	6.5	11.5	10.4
F-21	29.4	32.9	43.5	57.5	50.3	43.1	13.2	16.7	13.3
F-22	28.2	34.4	39.1	58.6	50.7	48.6	13.3	14.9	12.3
F-24	25.5	33.1	46.3	69.3	60.8	48.9	5.1	6	4.9
F-25	23.6	32.4	45.8	70.7	63.8	50.1	5.6	3.9	3.9
F-26	23.7	32.3	45.8	70.8	62.9	50.2	5.4	4.9	3.9
F-27	24.0	32.2	45.9	70.5	63.1	51.2	5.6	4.8	2.9
F-28	23.7	31.8	45.6	70.5	63.0	51.4	5.7	5.3	3.1
F-29	24.3	31.8	44.9	69.8	63.3	51.6	5.9	4.8	3.6
F-30	30.8	38.3	49.9	63.2	53.1	42.6	6.0	8.7	7.5
F-31	30.8	38	49.6	62.7	53.5	41.9	6.4	8.6	8.5
F-32	NA	NA	NA	NA	NA	NA	NA	NA	NA
F-33	NA	NA	NA	NA	NA	NA	NA	NA	NA
F-34	NA	NA	NA	NA	NA	NA	NA	NA	NA
F-35	26.2	32.9	42.1	67.4	57.7	49.3	6.5	9.3	8.8
F-36	26.5	32.9	42.4	67.6	57.8	49.2	6.0	9.3	8.4
F-37	27.6	32.1	41	65.9	56.2	47.6	6.5	11.5	11.3
F-38	26.8	33	42.4	67.0	57.6	49.3	6.2	9.4	8.3
F-39	23.4	28.2	36	67.1	56.4	49.9	9.4	15.5	14.1

Observation: Based on 7 days stress data, for % acidic of the F-10, F-20, F-21, F-22, and F-35 to F39 were comparable with the reference formulation (RMP);

For % Purity of the F-20, F-22 to F-29 and F-35 to F-39 were comparable with the reference formulation (RMP); and

For % Basic of the F-20, F-24 to F 31, F-35, F-37 & F-38 were comparable with the reference formulation (RMP).

E) SEC- HPLC

Table 7: SEC data (% Purity) of RMP & Formulation (F-10 to F-39) at 50˚ C

Buffers	Percentage Purity			Percentage HMW		
	0 D	**3 D**	**7 D**	**0 D**	**3 D**	**7 D**
RMP	99.2	NA	98.0	0.7	NA	1.4
F-10	98.9	95.5	97.8	0.9	1.8	2.5
F-11	98.9	98.3	96.9	1.1	1.5	2.3
F-19	99.0	97.9	96.4	0.9	1.8	3.0
F-20	99.4	98.6	97.9	0.6	1.1	1.5
F-21	99.1	98.3	97.3	0.8	1.5	2.1
F-22	99.1	98.3	97.0	0.8	1.5	2.3
F-24	97.8	97.4	96.4	2.2	2.4	2.7
F-25	98.9	98.0	96.9	1.1	1.8	2.3
F-26	98.9	97.9	96.9	1.1	1.8	2.4
F-27	98.8	98.0	97.0	1.1	1.8	2.3
F-28	99.6	98.7	97.8	1.1	1.8	2.2
F-29	98.9	97.9	97.1	1.1	1.9	2.3
F-30	99.2	98.2	97.1	0.7	1.4	2.2
F-31	99.2	98.1	96.8	0.7	1.6	2.4
F-32	97.3	95.9	95.0	2.6	3.7	4.3
F-33	96.7	95.4	94.7	3.2	4.3	4.9
F-34	97.2	NA	94.4	2.7	NA	4.9
F-35	99.5	98.6	97.7	0.5	1.0	1.5
F-36	99.5	98.4	97.3	0.5	1.3	2.1
F-37	99.4	98.6	97.9	0.6	1.1	1.5
F-38	99.5	98.6	97.8	0.5	1.1	1.6
F-39	99.2	98.1	97.1	0.7	1.7	2.2

Observation: Based on 7 days stress data the SEC data (% Basic) of the F10, F-20, F-35, F-37 & F-38 was comparable with the reference formulation (RMP); and

For % HMW of the F10, F-20, F-35, F-37 & F-38 was comparable with the reference formulation (RMP).

From the result of the above study, Formulation 20 (F-20) shown closer degradation compare to RMP.

EXAMPLE 3: STRESS STABILITY STUDY AT 40˚ C

Three different consistency batches BATCH 1, BATCH 2, and BATCH 3 of formulation F-20 given in table 1 was prepared to assess the stress stability. Following is the result from the stress stability study at 40˚ C.

A) Physical appearance:

All the samples were observed to be Colourless to yellow solution

B) pH:

Table 8: pH data of Stress Stability (40 ºC)

Buffers	pH				
Time Points	**0 D**	**7 D**	**14 D**	**21 D**	**28 D**
RMP	6.52	6.50	6.53	6.53	6.51
Batch 1	5.75	5.73	5.53	5.71	5.83
Batch 2	6.16	6.15	5.56	5.84	6.05
Batch 3	6.09	6.19	6.11	6.04	6.03

Observation: Based on 28 days Stress stability data the pH of all the 3 batches was comparable with the reference formulation (RMP) as depicted in Figure 1.

C) SEC- HPLC

Table 9: SEC data of Stress Stability (40 ºC)

Buffers	% HMW					% Purity					% LMW				
Time Points	**0D**	**7D**	**14D**	**21D**	**28D**	**0D**	**7D**	**14D**	**21D**	**28D**	**0D**	**7D**	**14D**	**21D**	**28D**
RMP	0.8	0.9	1.0	1.4	1.6	99.0	98.8	98.5	98.0	97.7	0.1	0.3	0.4	0.6	0.8
Batch 1	0.7	1.2	1.7	1.6	2.4	99.3	98.5	97.9	97.8	97.0	0	0.2	0.4	0.5	0.7
Batch 2	0.7	1.1	1.4	1.4	1.6	99.3	98.7	98.3	98.2	97.5	0	0.2	0.2	0.3	0.7
Batch 3	0.6	1.1	1.3	1.6	2.2	99.3	98.6	97.0	97.6	97.1	0.1	0.3	0.7	0.7	0.8

Observation: Based on 28 days Stress stability data the SEC- HPLC of all the 3 batches was comparable with the reference formulation (RMP) as depicted in Figure 2, 3 & 4.

D) CEX- HPLC

Table 10: CEX data of Stress Stability (40 ºC)

Buffers	% Acidic					% Purity					% Basic				
Time Points	**0D**	**7D**	**14D**	**21D**	**28D**	**0D**	**7D**	**14D**	**21D**	**28D**	**0D**	**7D**	**14D**	**21D**	**28D**
RMP	24.3	29.3	37.2	43.7	48	71.6	62.3	58.6	51.7	48.4	4.2	8.3	4.3	4.6	3.7
Batch 1	23.9	19.5	18.5	20.5	27.5	65.2	46.9	33.3	34.5	37.6	10.8	33.6	48.3	44.9	35
Batch 2	24.8	22.7	30.6	26.4	37.4	65.6	56.2	51.4	40.5	40.0	9.6	21.0	18.1	33.0	22.7
Batch 3	24.1	27.9	31.2	33.4	41.4	66.5	55.5	48.8	44.2	39.9	9.3	16.7	19.9	22.4	18.7

Observation: Based on 28 days Stress stability data the CEX- HPLC of all the 3 batches was comparable with the reference formulation (RMP) as depicted in Figure 5, 6 & 7.

EXAMPLE 4: REAL TIME (2˚ C -8˚ C) AND ACCELARATED TIME (25˚ C) DATA OF BATCH 4.

BATCH 4 of formulation F-20 given in table 1 was prepared to assess the real time and accelerated time stability. Following is the result from the real time and accelerated time stability study.

A. Physical appearance:

All the samples were observed to be Colourless to yellow solution

B. pH:

Table 11: pH data of Batch 4 at RT (2-8 ºC)

Buffers	**pH**	
Time Points	**0 D**	**3M**
Batch 4	6.08	6.25

Table 12: pH of Batch 4 at AT (25 ºC)

Buffers	**pH**		
Time Points	**0 D**	**1M**	**3M**
Batch 4	6.08	6.26	6.22

C. Protein Concentration:

Table 13: Protein Concentration data of Batch 4 at RT (2-8ºC)

Buffers	**Protein Concentration**	
Time Points	**0 D**	**3M**
Batch 4	165.90	185.21

Table 14: Protein Concentration data of Batch 4 at AT (25 ºC)

Buffers	**Protein Concentration**		
Time Points	**0 D**	**1M**	**3M**
Batch 4	165.90	168.60	180.65

D. SEC- HPLC

Table 15: SEC data of Batch 4 at RT (2-8 ºC)

Buffers	**% HMW**		**% Purity**		**% LMW**	
Time Points	**0 D**	**3M**	**0 D**	**3M**	**0 D**	**3M**
Batch 4	1.17	1.88	98.80	97.95	0.03	0.16

Table 26: SEC data of Batch 4 at AT (25 ºC)

Buffers	**% HMW**			**% Purity**			**% LMW**		
	0 D	**1 M**	**3 M**	**0 D**	**1 M**	**3 M**	**0 D**	**1 M**	**3 M**
Batch 4	1.17	1.82	2.27	98.8	98.06	97.25	0.03	0.13	0.48

E. CEX- HPLC

Table 17: CEX data of Batch 4 at RT (2-8 ºC)

Buffers	**% Acidic**		**% Purity**		**% Basic**	
Time Points	**0 D**	**3M**	**0 D**	**3M**	**0 D**	**3M**
Batch 4	23.93	26.32	66.93	64.56	9.12	9.14

Table 18: CEX data of Batch 4 at AT (25 ºC)

Buffers	**% Acidic**			**% Purity**			**% Basic**		
	0 D	**1 M**	**3 M**	**0 D**	**1 M**	**3 M**	**0 D**	**1 M**	**3 M**
Batch 4	23.93	28.73	32.43	66.93	57.65	52.76	9.12	13.61	14.80

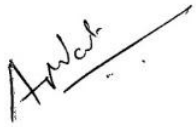
Result of the above study indicated that there is no change between formulation F-20 of present invention and RMP formulation. This means that formulation of present invention is highly comparable with the RMP formulation.

We Claim,

1. A stable liquid pharmaceutical formulation comprising: anti-α4β7 antibody, buffer, stabilizer, anti-oxidant, viscosity reducer and surfactant.
2. The stable liquid pharmaceutical formulation of claim 1, wherein buffer is Histidine/ histidine monohydrochloride, having concentration of 0-100 mM, more preferably about 50 mM.
3. The stable liquid pharmaceutical formulation of claim 1, wherein stabilizer is sucrose, having concentration of 0-100 mg/ml, more preferably about 45 mg/ml.
4. The stable liquid pharmaceutical formulation of claim 1, wherein anti-oxidant is L-methionine, having concentration of 0-2 mg/ml, more preferably about 1.49 mg/ml.
5. The stable liquid pharmaceutical formulation of claim 1, wherein viscosity reducer is L-lysine HCl, having concentration of 0-10 mg/ml, more preferably about 8 mg/ml.
6. The stable liquid pharmaceutical formulation of claim 1, wherein surfactant is polysorbate 80, having concentration of 0-2 mg/ml, more preferably about 2 mg/ml.
7. A stable liquid pharmaceutical formulation of Vedolizumab comprising: Histidine/ histidine monohydrochloride as buffer, sucrose as stabilizer, L- lysine HCl as viscosity reducer, L- methionine as anti-oxidant, polysorbate 80 as surfactant, wherein the formulation has the molar ratio of sugar to anti-α4β7 antibody (mole:mole) less than 150:1.
8. The stable liquid pharmaceutical formulation of claim 7, wherein the formulation comprises: 0-100 mM histidine/ histidine monohydrochloride, 0-100 mg/ml sucrose, 0-10 mg/ml L-lysine HCl, 0-2 mg/ml L-methionine and 0-2 mg/ml polysorbate 80, wherein pH of formulation is about 5.8 to 6.8.
9. The stable liquid pharmaceutical formulation of claim 7, wherein the formulation comprises: 160 mg/ml Vedolizumab, 50 mM histidine/ histidine HCl, 45 mg/ml sucrose, 8 mg/ml L-lysine HCl, 1.49 mg/ml L-methionine, and 2 mg/ml polysorbate 80, at pH 5.8 to 6.8.
10. A stable liquid pharmaceutical formulation of 160 mg/ml Vedolizumab comprising: 50 mM Histidine/ histidine monohydrochloride as buffer, 45 mg/ml sucrose as stabilizer, 8 mg/ml L- lysine HCl as viscosity reducer, 1.49 mg/ml L- methionine as anti-oxidant, 2

mg/ml polysorbate 80 as surfactant, wherein the formulation has the molar ratio of sugar to anti-α4β7 antibody (mole:mole) less than 150:1.

Dated: 27th June, 2023



Signature: _____

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For and on behalf of the applicant

To,

The Controller of Patent

The Patent Office

At Mumbai.

ABSTRACT

STABLE LIQUID FORMULATION OF AN ANTI-α4ß7 ANTIBODY

The invention provides stable liquid formulation of anti-α4ß7 antibody comprising buffer, bulking agent/ stabilizer, viscosity reducer, antioxidant and surfactant, wherein the bulking agent/ stabilizer is sucrose, viscosity reducer is L-lysine HCl and antioxidant is methionine. The stable liquid formulation according to present invention is suitable for subcutaneous administration.