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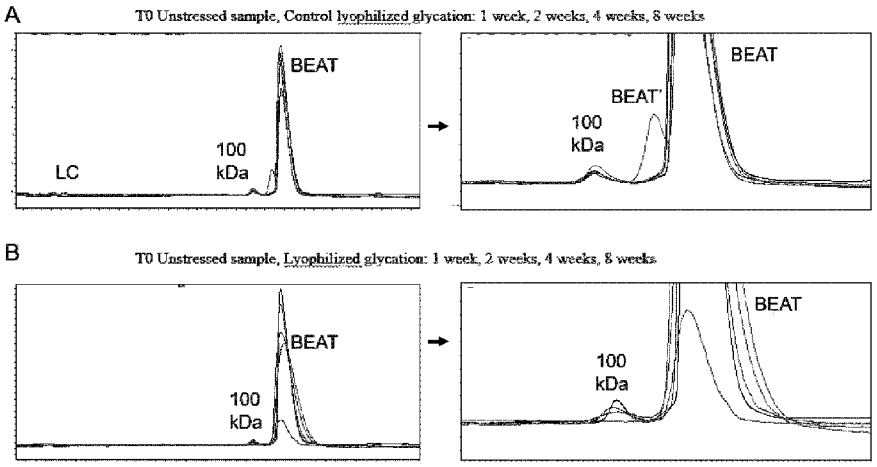
(54) Title: COMPOSITIONS COMPRISING A BISPECIFIC ANTIBODY, BUFFFER AND ONE OR MORE STABILIZING AGENTS

FIG. 24



(57) Abstract: The present invention relates to stable formulations of a therapeutic antibody; preferably the therapeutic antibody is a monoclonal bispecific antibody.

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COMPOSITIONS COMPRISING A BISPECIFIC ANTIBODY, BUFFFER AND ONE OR MORE STABILIZING AGENTS

TECHNICAL FIELD

The present invention relates to stable formulations of a therapeutic antibody; preferably the therapeutic antibody is a monoclonal bispecific antibody.

BACKGROUND

The pharmaceutical formulation of a therapeutic product commonly comprises, besides an active pharmaceutical ingredient (API) with therapeutic properties, also inactive ingredients, or excipients that contribute to the stability, bioavailability and effectiveness of the API.

Example of excipients include solvents, diluents, buffering agents, pH-adjusting agents, surfactants, preservatives, tonicifying agents, antioxidants etc. Appropriate excipients are chosen based on the drug product presentation, on the route of administration and on the administration dosage, as well as based on their effect on the stability of the API.

Biological molecules, like proteins, i.e. antibodies, are nowadays developed as therapeutics to treat a variety of diseases spanning from cancer to autoimmune diseases. To use a biological molecule for therapeutic purposes, the full comprehension of its stability is necessary to elaborate proper formulation and package as well as to provide proper storage conditions and shelf life, essential for regulatory authorities. At this aim, analytical tests are carried out to assess the stability of the pharmaceutical formulation when subjected to different stresses (i.e. freeze-thaw cycles, shaking) and in different conditions (i.e. short term and long term storage, storage at different temperatures). Different tests are required to prove biomolecule stability, including visual inspection, sub-visible particle analysis, aggregate formation investigation etc. Also, a way to explore the stability of a biological molecule consists in forcing its degradation using different kinds of physical and chemical stress; these investigations are known as Forced Degradation Studies (FDS) or stress studies. For the production of therapeutic antibodies, the development of stable pharmaceutical formulations is necessary to obtain an effective and stable drug products, stable formulations are therefore a primary need of the pharmaceutical industry.

DESCRIPTION

The present invention relates to a stable pharmaceutical formulation comprising a bispecific antibody or an antibody fragment thereof, a buffer and one or more stabilizing or tonicity agents.

In one aspect of the present invention the pharmaceutical formulation is liquid, or lyophilized or reconstituted.

According to another aspect of the present invention, the pharmaceutical formulation comprises an antibody or antibody fragment thereof is present within said pharmaceutical formulation at a concentration between 0.05 mg/mL and 15 mg/mL.

According to another aspect of the present invention, the disclosed pharmaceutical formulation pharmaceutical formulation has a pH between 5.5 and 7.0.

In a further aspect, the pharmaceutical formulation comprises a buffer is selected from the group comprising acetate, L-histidine, citrate and phosphate.

In a more specific aspect, the buffer is histidine, present within said pharmaceutical formulation at a concentration comprised between 1 mM and 20 mM.

In another aspect, the pharmaceutical formulation according to the present invention comprises one or more stabilizing or tonicity agent is selected from the group comprising sodium acetate, sodium bicarbonate, sodium carbonate, sodium chloride, potassium acetate, potassium bicarbonate, potassium carbonate, potassium chloride, calcium chloride, sucrose, glutamate, mannitol, polyols, Polysorbate 20, Polysorbate 40, Polysorbate 80, Poloxamer, Poloxamer 188, Poloxamer 407, amino acids such as histidine, arginine, glycine, methionine, proline, lysine, glutamic acid, amines, cyclodextrins, β-cyclodextrins, polyvinylpyrrolidone, polyethylene glycol 400, sorbitol, trehalose and EDTA, present within said pharmaceutical formulation at a percentage between about 0.005% and about 20%.

In a particular aspect, the stabilizing or tonicity agent is sucrose present within said pharmaceutical formulation at a concentration comprised between about 2% and about 15%; and/or mannitol present within said pharmaceutical formulation at a concentration comprised between about 2% and about 6%; and/or glycine present within said pharmaceutical formulation at a concentration comprised between about 0.4% and about 1.2%; and/or Polysorbate 80 present within said pharmaceutical formulation at a concentration comprised between about 0.01% and about 0.11%; and/or arginine present within said pharmaceutical formulation at a concentration of about 0.4%; and/or glutamate present within said pharmaceutical formulation at a concentration of about 0.4%; and/or polyvinylpyrrolidone present within said pharmaceutical formulation at a concentration of about 0.1%; and/or β-cyclodextrin present within said pharmaceutical formulation at a concentration of about 0.1%.

In one embodiment of the present invention, the pharmaceutical formulation comprises a bispecific antibody or fragment thereof present within said pharmaceutical formulation at a concentration selected from the group comprising about 0.1 mg/mL, about 0.3 mg/mL , about 1 mg/mL and about 10±2 mg/mL, histidine buffer present within said pharmaceutical formulation at a concentration of about 5 mM, sucrose present within said pharmaceutical formulation at a percentage of about 5% and Polysorbate 80 present within said pharmaceutical formulation at a percentage of about 0.02%, and wherein said pharmaceutical formulation has pH of about 6.3.

In a particular embodiment, the bispecific antibody or fragment thereof is present within said pharmaceutical formulation at a concentration of about 10±2 mg/mL and the pharmaceutical formulation is liquid.

In another particular embodiment, the bispecific antibody or fragment thereof is present within said pharmaceutical formulation at a concentration of about 0.1 mg/mL and the pharmaceutical formulation is lyophilized or reconstituted.

According to one aspect of the present invention, the pharmaceutical formulation is stable at about 25°C for at least 1 month; and/or at about 5°C for at least 1 month; and/or at about -20°C for at least 48 months; at about -60°C for at least 48 months; and/or at about -80°C for at least 36 months.

According to another aspect, the pharmaceutical formulation is stable at about 40°C for at least 3 months; and/or at about 25°C for at least 24 months; and/or at about 5°C for at least 36 months.

In one embodiment of the present invention, the disclosed pharmaceutical formulation comprises a bispecific antibody or an antibody fragment thereof that binds CD3 and HER2. In a more particular aspect, the bispecific antibody or an antibody fragment comprises the amino acid sequences of SEQ ID NOs: 2, 4 and 6.

The present invention also relates a method of manufacturing the pharmaceutical formulation of any one of the preceding claims.

Disclosed by the present invention is also a method to test the pharmaceutical formulation of any one of the preceding claims to any assay for stability determination.

The present invention also discloses an article of manufacture comprising the pharmaceutical formulation of any one of the preceding claims.

The present invention relates to a stable pharmaceutical formulation comprising a bispecific antibody or an antibody fragment thereof, a buffer and one or more stabilizing or tonicity agents.

As used herein, the following terms have the following meanings: "a", "an", and "the" as used herein refers to both singular and plural unless the context clearly dictates otherwise.

Unless otherwise defined, scientific and technical terms used in connection with the present invention shall have the meanings that are commonly understood by those of ordinary skill in the art. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. Generally, nomenclatures utilized in connection with, and techniques of cell and tissue culture, molecular biology, and protein and oligo- or polynucleotide chemistry, laboratory procedures and techniques of analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well-known and commonly used in the art.

Temperatures above 0°C are expressed in the present invention either by preceding the temperature value by “+” or not, e.g. 25°C and +25°C can be used interchangeably according to the present invention.

According to one aspect of the present invention, the disclosed pharmaceutical formulation may be liquid, or lyophilized or reconstituted.

A "liquid" formulation is one that has been prepared in a liquid format. Such a formulation may be suitable for direct administration to a subject or, alternatively, can be packaged for storage either in a liquid form, in a frozen state or in a dried form (e.g. lyophilized) for later reconstitution into a liquid form or other forms suitable for administration to a subject.

A "lyophilized" formulation is one that has been prepared by freeze-drying a liquid or pre- lyophilization formulation. Freeze-drying is performed by freezing the formulation and then subliming ice from the frozen content at a temperature suitable for primary drying. Under this condition the product temperature is below the collapse temperature of the formulation. A secondary drying stage may then be carried out, which produces a suitable lyophilized cake.

A "reconstituted" formulation is one that has been prepared by dissolving a lyophilized protein formulation in a diluent such that the protein is dispersed in the reconstituted formulation. The reconstituted formulation should be suitable for administration (e.g. parenteral administration) to a subject to be treated with the protein of interest.

Suitable "reconstitution media" useful for the preparation of a reconstituted formulation include ones which are pharmaceutically acceptable (safe and non-toxic for administration to a human). Examples of suitable diluents include sterile water for injection (SWFI), bacteriostatic water for injection (BWFI). Water for injection (WFI), a pH buffered solution e.g. phosphate-buffered saline (PBS), sterile saline solution, Ringer's solution or dextrose solution and the buffer used to prepare the pharmaceutical formulation.

According to one embodiment of the present invention, lyophilization is carried out by at least one lyophilization cycle, comprising the steps of precooling, freezing/annealing, primary drying and secondary drying. In particular, according to an embodiment of the present invention, precooling is performed at a temperature comprised between about 0°C and about 10°C, preferably at a temperature of about 5°C, for a ramp time, namely the time require to change temperature from one step to other, comprised between about 5 min and about 60 min, preferably between about 10 min and about 40 min, more preferably for a ramp time of about 20 min, and for an hold time, namely the time in which the sample is kept at the defined temperature, comprised between about 5 hours and about 12 hours, preferably between about 7 hours and about 10.30 hours, more preferably for an hold time of about 7 hours. Freezing/annealing is performed by a first freezing step at a temperature comprised between about -5°C and about 5°C, preferably at a temperature of about 0°C for a ramp time comprised between about 1 min and about 55 min, preferably between about 2 min and about 10 min, more preferably for a ramp time of about 5 min, and for an hold time comprised between about 20 min and about 40 min, preferably for an hold time of about 30 min; followed by a second freezing step at a temperature comprised between about -40°C and about -50°C, preferably at a temperature of about -45°C, for a ramp time comprised between about 15 min and about 2 hours, preferably between about 20 min and about 1.40 hours, more preferably for a ramp time of about 45 min, and for an hold time comprised between about 1 hour and about 3 hours, preferably for an hold time of about 2 hours; followed by a third freezing step at a temperature comprised between about -5°C and about -25°C, preferably at a temperature of about -15°C, for a ramp time comprised between about 5 min and about 1.30 hours, preferably between about 15 min and about 1.10 hours, more preferably for a ramp time of about 30 min, and for an hold time comprised between about 1 hour and about 8 hours, preferably between about 2 hours and about 6 hours, more preferably for an hold time of about 2 hours; followed by a forth freezing step at a temperature comprised between about -40°C and about -50°C, preferably at a temperature of about -45°C, for a ramp time comprised between about 5 min and about 1.30 hours, preferably between about 15 min and about 1.10 hours, more preferably for a ramp time of about 30 min, and for an hold time comprised between about 1 hour and about 3 hours, preferably for an hold time of about 2 hours. Primary drying is performed at a temperature

comprised between about -30°C and about -40°C, preferably at a temperature of about -35°C for a ramp time of about 1 hour and a hold time of about 120 hours with vacuum levels between XX and YY, preferable at a vacuum level of XX. Secondary drying is performed at a temperature comprised between about 20°C and about 30°C, preferably at a temperature of about 25°C for a ramp time of about 20 hours and an hold time of about 10 hours with or without the application of vacuum at upto the same level as primary drying. The present invention also comprises lyophilization processes at any value of the temperature, the ramp time and the hold time between the above cite values.

The term "buffer" as used herein refers to a buffered solution that resists changes in pH by the action of its acid-base conjugate components. A buffer of this invention has a pH in the range from about 5.0 to about 7.0; preferably the buffer has a pH selected from the group comprising pH 6, pH 6.3 and pH 6.5. Examples of buffers that can control the pH in this range include acetate (e.g. sodium acetate), succinate (such as sodium succinate), gluconate, amino acids, such as histidine (e.g. histidine-HCl), citrate, phosphate, other organic acid buffer, their salts and combinations of buffers. In one embodiment of the present invention the buffer is present within the pharmaceutical formulation at concentration between about 1 mM and about 20 mM; preferably the buffer is present within the pharmaceutical formulation at concentration selected from the group comprising 1 mM, 3 mM, 5 mM, 7 mM, 10 mM, 13mM, 15 mM, 17 mM and 20 mM. The present invention also includes a buffer with a concentration at any intermediate value of the above said values. In a particular embodiment of the present invention, the buffer is Histidine, present within the pharmaceutical formulation at a concentration of about 5 mM.

One or more stabilizing or tonicity agent may be added to the formulation to stabilize the protein in the lyophilized form. Said stabilizing or tonicity agent is selected from the group comprising sodium acetate, sodium bicarbonate, sodium carbonate, sodium chloride (NaCl), potassium acetate, potassium bicarbonate, potassium carbonate, potassium chloride, calcium chloride (CaCl2), glutamate, sugars such as sucrose, glucose and trehalose, polyols such as mannitol, maltitol, sorbitol, xylitol, erythritol, and isomalt, polyethylene glycol, such as PEG400, Ethylenediaminetetraacetic acid (EDTA), amino acids such as histidine (e.g. histidine-HCl), arginine (e.g. arginine hydrochloride) and glycine, methionine, proline, lysine (e.g. lysine-HCl), glutamic acid, glutamine, cysteine, amines, glutathione, cyclodextrin, such as such as Hydroxypropyl β-cyclodextrin (HPBCD), Hydroxypropyl-sulfobutyl β-cyclodextrin (HPSBCD), Sulfobutylether β-cyclodextrin (SBECD), β-cyclodextrin (BetaCD), α-cyclodextrin (Alpha CD) and γ-cyclodextrin (Gamm CD) and surfactants. Non limiting examples of a typical surfactant include: non- ionic surfactants (HLB 6 to 18) such as sorbitan fatty acid esters (e.g. sorbitan monocaprylate, sorbitan

monolaurate, sorbitan monopalmitate), glycerine fatty acid esters (e.g. glycerine monocaprylate, glycerine monomyristate, glycerine monostearate), poly glycerine fatty acid esters (e.g. decaglyceryl monostearate, decaglyceryl distearate, decaglyceryl monolinoleate), polyoxyethylene sorbitan fatty acid esters (e.g. polyoxyethylene sorbitan monolaurate, polyoxyethylene sorbitan monooleate, polyoxyethylene sorbitan monostearate, polyoxyethylene sorbitan monopalmitate, polyoxyethylene sorbitan trioleate, polyoxyethylene sorbitan tristearate), polyoxyethylene sorbitol fatty acid esters (e.g. polyoxyethylene sorbitol tetrastearate, polyoxyethylene sorbitol tetraoleate), polyoxyethylene glycerine fatty acid esters (e.g. polyoxyethylene glyceryl monostearate), polyethylene glycol fatty acid esters (e.g. polyethylene glycol distearate), polyoxyethylene alkyl ethers (e.g. polyoxyethylene lauryl ether), polyoxy ethylene polyoxypropylene alkyl ethers (e.g. polyoxyethylene polyoxypropylene glycol ether, polyoxyethylene polyoxypropylene propyl ether, polyoxyethylene polyoxypropylene cetyl ether), polyoxyethylene alkylphenyl ethers (e.g. polyoxyethylene nonylphenyl ether), polyoxyethylene hydrogenated castor oils (e.g. polyoxyethylene castor oil, polyoxyethylene hydrogenated castor oil), polyoxyethylene beeswax derivatives (e.g. polyoxyethylene sorbitol beeswax), polyoxyethylene lanolin derivatives (e.g. polyoxyethylene lanolin), and polyoxyethylene fatty acid amides (e.g. polyoxyethylene stearyl amide); anionic surfactants such as Cio-Cis alkyl sulfates salts (e.g. sodium cetyl sulfate, sodium lauryl sulfate, sodium oleyl sulfate), polyoxyethylene Cio-Cis alkyl ether sulfates salts with an average of 2 - 4 moles of ethylene oxide (e.g. sodium polyoxyethylene lauryl sulfate), and Cs-Cis alkyl sulfosuccmate ester salts (e.g. sodium lauryl sulfosuccmate ester); natural surfactants such as lecithin, glycerophospho lipid, sphingophospho lipids (e.g. sphingomyelin) and sucrose esters of C 12-C 18f atty acids; Poloxamers such as Poloxamer 188, Poloxamer 407, Poloxamer 124, Poloxamer 237, Poloxamer 338, polyvinylpyrrolidone, salts and combinations of the above cited components. Preferably, the surfactant is selected from polyoxyethylene sorbitan fatty acid esters. Preferably, the surfactant is selected from polyoxyethylene sorbitan fatty acid esters. Particularly preferably the surfactant is Polysorbate 20, 21 , 40, 60, 65, 80, 81 and 85, most preferably Polysorbate 80. Polysorbate 80 is also known by the brand name Tween 80™ (ICI Americas, Inc.).

In particular embodiments of the present invention, the stabilizing or tonicity agent is selected from the group comprising sodium acetate, sodium bicarbonate, sodium carbonate, sodium chloride, potassium acetate, potassium bicarbonate, potassium carbonate, potassium chloride, calcium chloride, sucrose, glutamate, mannitol, polyols, Polysorbate 20, Polysorbate 40, Polysorbate 80, Poloxamer, Poloxamer 188, Poloxamer 407, amino acids such as histidine, arginine, glycine, methionine, proline, lysine, glutamic acid,

amines, cyclodextrins, β-cyclodextrins, polyvinylpyrrolidone, polyethylene glycol 400, sorbitol, trehalose and EDTA.

In a more particular embodiment, the stabilizing or tonicity agent is present within the pharmaceutical formulation at a percentage between about 0.005% and about 20%. More in particular, sucrose is present within said pharmaceutical formulation at a concentration comprised between about 0.1% and about 20%, preferably between about 2% and about 15%,more preferably at a concentration selected from the group comprising 2%, 4%, 6%, 8%, 10%, 12%, 14% and 15%; and/or mannitol is present within said pharmaceutical formulation at a concentration comprised between about 0.5% and about 10%, preferably between about 2% and about 6%, more preferably at a concentration selected from the group comprising 2%, 2.5%, 4%, 5% and 6%; and/or glycine is present within said pharmaceutical formulation at a concentration comprised between about 0.1% and about 2%, preferably between about 0.4% and about 1.2%, more preferably at a concentration selected from the group comprising 0.4%, 0.5%, 0.8%, 1%, 1.2% and 2%; and/or Polysorbate 80 is present within said pharmaceutical formulation at a concentration comprised between about 0.001% and about 1%, preferably between about 0.01% and about 0.11%, more preferably at a concentration selected from the group comprising 0.01%; 0.02%, 0.05%, 0.06% and 0.11%; and/or arginine is present within said pharmaceutical formulation at a concentration between about 0.1% and about 1%, preferably of about 0.4%; and/or glutamate is present within said pharmaceutical formulation at a concentration between about 0.1% and about 1%, preferably of about 0.4%; and/or polyvinylpyrrolidone (PVP) is present within said pharmaceutical formulation at a concentration between about 0.05% and about 0.1%, preferably of about 0.1%; and/or β-cyclodextrin is present within said pharmaceutical formulation at a concentration between about 0.05% and about 0.1%, preferably of about 0.1%. The present invention also includes stabilizing or tonicity agents with a concentration at any intermediate value of the above said values.

The pharmaceutical formulation according to the present invention also comprises a bispecific antibody or an antibody fragment thereof.

The term "antibody" or “immunoglobulin” are herein used interchangeably and referred to whole antibodies and any antigen binding fragments or single chains thereof. An "antibody" refers to a glycoprotein comprising at least two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds, or an antigen binding fragment thereof. Each heavy chain is comprised of a heavy chain variable region (abbreviated herein as VH) and a heavy chain constant region. The heavy chain constant region is comprised of three domains, CH1, CH2 and CH3. Each light chain is comprised of a light chain variable

region (abbreviated herein as VL) and a light chain constant region. The light chain constant region is comprised of one domain, CL. The VH and VL regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR) with are hypervariable in sequence and/or involved in antigen recognition and/or usually form structurally defined loops, interspersed with regions that are more conserved, termed framework regions (FR or FW). Each VH and VL is composed of three CDRs and four FWs, arranged from amino- terminus to carboxy- terminus in the following order: FW1, CDR1, FW2, CDR2, FW3, CDR3 and FW4. The amino acid sequences of FW1, FW2, FW3, and FW4 all together constitute the "non-CDR region" or "non-extended CDR region" of VH or VL as referred to herein. Antibodies are grouped into classes, also referred to as isotypes, as determined genetically by the constant region. Human constant light chains are classified as kappa (CK) and lambda (CX) light chains. Heavy chains are classified as mu (μ), delta (δ), gamma (γ), alpha (a), or epsilon (ε), and define the antibody's isotype as IgM, IgD, IgG, IgA, and IgE, respectively. Thus, "isotype" as used herein is meant any of the classes and/or subclasses of immunoglobulins defined by the chemical and antigenic characteristics of their constant regions. The known human immunoglobulin isotypes are IgG1 (IGHG1), IgG2 (IGHG2), IgG3 (IGHG3), IgG4 (IGHG4), IgA1 (IGHA1), IgA2 (IGHA2), IgM (IGHM), IgD (IGHD), and IgE (IGHE). The IgG class is the most commonly used for therapeutic purposes. In humans this class comprises subclasses IgG1, IgG2, IgG3 and IgG4.

Antibody fragments include, but are not limited to, (i) the Fab fragment consisting of VL, VH, CL and CHI domains, including Fab' and Fab'-SH, (ii) the Fd fragment consisting of the VH and CHI domains, (iii) the Fv fragment consisting of the VL and VH domains of a single antibody; (iv) the dAb fragment (Ward ES et al., (1989) Nature, 341 : 544-546) which consists of a single variable, (v) F(ab')2 fragments, a bivalent fragment comprising two linked Fab fragments (vi) single chain Fv molecules (scFv), wherein a VH domain and a VL domain are linked by a peptide linker which allows the two domains to associate to form an antigen binding site (Bird RE et al, (1988) Science 242: 423-426; Huston JS et al, (1988) Proc. Natl. Acad. Sci. USA, 85: 5879-83), (vii) bispecific single chain Fv dimers (PCT/US92/09965), (viii) "diabodies" or "triabodies", multivalent or multispecific fragments constructed by gene fusion (Tomlinson I & Hollinger P (2000) Methods Enzymol. 326: 461-79; WO94/13804; Holliger P et al, (1993) Proc. Natl. Acad. Sci. USA, 90: 6444-48) and (ix) scFv genetically fused to the same or a different antibody (Coloma MJ & Morrison SL (1997) Nature Biotechnology, 15(2): 159-163).

In one embodiment of this invention, the disclosed antibody is monoclonal.

The term "monoclonal antibody" as used herein, refers to antibodies that are produced by clone cells all deriving from the same single cell, and that specifically bind the same epitope of the target antigen. When therapeutic antibodies are produced, the generation of monoclonal antibodies is preferred over polyclonal antibodies. In fact, while monoclonal antibodies are produced by cells originating from a single clone and bind all the same epitope, polyclonal antibodies are produced by different immune cells and recognize multiple epitopes of a certain antigen. Monoclonal antibodies assure batch to batch homogeneity, reduced cross-reactivity and high specificity toward the target. Monoclonal antibodies can be expressed, for instance in host cells, using recombinant DNA, giving rise to a recombinant antibody.

In a certain embodiment of this invention the antibody is a recombinant antibody.

The term "recombinant antibody" as used herein, refers to an antibody that has been produced by any process involving the use of recombinant DNA. A recombinant antibody can be engineered in such a way to improve characteristics such as immunogenicity, binding affinity, molecular size, specificity, half-life, and format. Examples of recombinant antibodies include, but are not limited to engineered antibodies, chimeric antibodies, CDRs grafted antibodies (such as humanized antibodies), fully human antibodies, antibody fragments, Fc-engineered antibodies, multispecific antibody (such as bispecific, trispecific, tetraspecific antibody), monomeric and multimeric antibodies (such as homo-dimeric and hetero-dimeric antibodies).

In a more specific embodiment of the antibody of the present invention is a hetero-dimeric antibody.

The term "hetero-dimeric antibody" or "hetero-dimeric fragment" or "hetero-dimer" as used herein includes an immunoglobulin molecule or part of comprising at least a first and a second polypeptide, like a first and a second domain, wherein the second polypeptide differs in amino acid sequence from the first polypeptide. Preferably, a hetero-dimeric immunoglobulin comprises two polypeptide chains, wherein the first chain has at least one non identical domain to the second chain, and wherein both chains assemble, i.e. interact through their non-identical domains. Specifically, a hetero-dimeric immunoglobulin comprises at least two domains, wherein the first domain is non identical to the second domain, and wherein both domains assemble, i.e. interact through their protein-protein interfaces. More preferably the hetero-dimeric immunoglobulin, has binding specificity for at least two different ligands, antigens or binding sites, i.e. is bispecific.

The term "bispecific antibody" refers to any antibody having two binding sites that can bind two different epitopes of the same antigen, or two different antigens.

According to one aspect of the present invention, the antibody or antibody fragment thereof is present within said pharmaceutical formulation at a concentration between 0.05 mg/mL and 15 mg/mL, more in particular the concentration selected from the group comprising about 0.1 mg/mL, about 0.3 mg/mL , about 1 mg/mL and about 10±2 mg/mL. The present invention also includes antibody or antibody fragment thereof present within the disclosed pharmaceutical formulation at a concentration at any intermediate value of the above said values.

According to a particular embodiment of the present invention, the pharmaceutical formulation comprises a bispecific antibody or fragment thereof present within said pharmaceutical formulation at a concentration selected from the group comprising about 0.1 mg/mL, about 0.3 mg/mL, about 1 mg/mL and about 10±2 mg/mL, histidine buffer present within said pharmaceutical formulation at a concentration of about 5 mM, sucrose present within said pharmaceutical formulation at a percentage of about 5% and Polysorbate 80 present within said pharmaceutical formulation at a percentage of about 0.02%, and wherein said pharmaceutical formulation has pH of about 6.3.

According to a more particular embodiment of the present invention, the pharmaceutical formulation comprises a bispecific antibody or fragment thereof present within said pharmaceutical formulation at a concentration of about 10±2 mg/mL, histidine buffer present within said pharmaceutical formulation at a concentration of about 5 mM, sucrose present within said pharmaceutical formulation at a percentage of about 5% and Polysorbate 80 present within said pharmaceutical formulation at a percentage of about 0.02%, and wherein said pharmaceutical formulation has pH of about 6.3, and said pharmaceutical formulation is liquid.

According to another particular embodiment of the present invention, the pharmaceutical formulation comprises a bispecific antibody or fragment thereof present within said pharmaceutical formulation at a concentration of about 0.1 mg/mL, histidine buffer present within said pharmaceutical formulation at a concentration of about 5 mM, sucrose present within said pharmaceutical formulation at a percentage of about 5% and Polysorbate 80 present within said pharmaceutical formulation at a percentage of about 0.02%, and wherein said pharmaceutical formulation has pH of about 6.3, and said pharmaceutical formulation is lyophilized or reconstituted.

In the present invention, the hetero-dimeric bispecific antibody may be generated by BEAT® technology (WO2012131555). In one embodiment of this invention, the bispecific antibody, referred to as BEAT®1, binds the cluster of differentiation 3 (CD3) expressed by T-cells and the human epidermal growth factor

receptor 2 (HER2), often overexpressed in breast cancer cells. BEAT®1 according to the present invention comprises the amino acid sequences of SEQ ID NOs: 1 to 6.

The pharmaceutical formulation according to the present invention is stable. A "stable" formulation is one in which the protein therein essentially retains its physical stability and/or chemical stability and/or biological activity upon storage. Various analytical techniques for measuring protein stability are available in the art and are reviewed for example in Peptide and Protein Drug Delivery, 247-301, Vincent Lee Ed., Marcel Dekker, Inc., New York, New York, Pubs. (1991) and Jones A (1993) Adv Drug Delivery Rev, 10: 29-90. Stability can be measured at a selected temperature for a selected time period.

To use biological molecules, such as antibody, as therapeutics, it is necessary to investigate the stability of the drug product, analytical tests useful to determine said stability include but are not limited to: monitoring of the visual appearance as a significant change in the appearance of sample may indicate product degradation and/or microbial contamination leading to safety risk for the patients; sub-visible particles analysis, as the presence of higher sub-visible particles in parental solutions may lead to immunogenic responses; protein content measurement (e.g. by measuring absorbance at 280 nm wavelength (A280) by UV-VIS Spectroscopy or by SoloVPE) as any significant variation from its target concentration would not provide effective dose to patients; pH measurement as changes in pH may be indicative of degradation of buffering agents and lead to protein instability; size variants monitoring (e.g. by SE-HPLC and/or by cGE reduced and non-reduced) as changes in monomeric content toward aggregates (higher size than monomer) or fragments (smaller size than monomer) is an indication of its degradation; charge variants monitoring (e.g. by cIEF) as changes in content of charged variants is an indication of its degradation; antibody potency measurement (e.g. by ELISA) as any significant change of binding property of the antibody toward its target would indicate antibody degradation. Additionally, the amino acid sequence as well as post-translational modifications (i.e. deamidation, oxidation, glycation, N-terminal variants, C-terminal variants and glycosylation site occupancy) can be verified, for instance by peptide mapping. Other characteristics of the formulation can be monitored, such as osmolarity and viscosity, as well as the protein thermal stability for instance by nano-format of Differential Scanning Fluorimetry (DSF)Additionally, International Conference on Harmonization (ICH) guidance imposes to perform Forced Degradation Studies (FDS) of new biological entities in phase III clinical trials at the latest. FDS enables to recognize how the BDS and DP will react to different stresses and time. In that respect, it is recommended to test the molecules under a range of conditions such as acid and base hydrolysis, thermal degradation, photolysis, oxidation and freeze-thaw cycles and shear may be included. Non

limiting examples of stresses useful to degrade the molecule comprise: elevated temperature (Markus Haberger & al, 2014) which induces levels of degradation (aggregation, fragmentation); oxidation (Christine Nowak & al, 2017; Emilien Folzer & al, 2015) as Reactive Oxygen Species (ROS) can be generated through different mechanisms which affects various amino acids; acidic and basic pH (Erinc Sahin & al, 2011; Christine Nowak & al, 2017; Markus Haberger & al, 2014) which has been conducted in combination with thermal stress as low temperatures allows for comparison from stability studies and high temperatures accelerate degradation pathways; light exposure (Pei Qi & al, 2008) which can lead to oxidation of amino acids residues as well as formation of aggregation; forced glycation with reducing sugars resulting in glycation of the surface-exposed lysine residues which could affect binding and efficacy; freeze-thaw (Manasi Puri & al, 2015; Xavier Le Saout & al, 2010) to determine the susceptibility of the molecule to controlled temperature cycling (Freeze-thaw data are not included in the report). Enzymatic digestion can also be performed in order to better understand the molecule. The degraded material can thus be generated as part of a FDS (Elnaz Tamizi & al, 2015; Andrea Hawe & al, 2012; Christine P. Chan, 2016).

According to a more particular embodiment of the present invention, the liquid pharmaceutical formulation comprising a bispecific antibody or fragment thereof present within said pharmaceutical formulation at a concentration of about 10±2 mg/mL, histidine buffer present within said pharmaceutical formulation at a concentration of about 5 mM, sucrose present within said pharmaceutical formulation at a percentage of about 5% and Polysorbate 80 present within said pharmaceutical formulation at a percentage of about 0.02%, and wherein said pharmaceutical formulation has pH of about 6.3, is stable at about 25°C for at least 1 month; and/or at about 5°C for at least 1 month; and/or at about -20°C for at least 48 months; at about -60°C for at least 48 months; and/or at about -80°C for at least 36 months.

According to another particular embodiment of the present invention, the lyophilized or reconstituted pharmaceutical formulation comprising a bispecific antibody or fragment thereof present within said pharmaceutical formulation at a concentration of about 0.1 mg/mL, histidine buffer present within said pharmaceutical formulation at a concentration of about 5 mM, sucrose present within said pharmaceutical formulation at a percentage of about 5% and Polysorbate 80 present within said pharmaceutical formulation at a percentage of about 0.02%, and wherein said pharmaceutical formulation has pH of about 6.3, is stable at about 40°C for at least 3 months; and/or at about 25°C for at least 24 months; and/or at about 5°C for at least 36 months.

In a certain embodiment, the present invention relates to a pharmaceutical formulation for use in the treatment of cancer characterized by the overexpression of HER2 and in particular selected from the group comprising breast, ovarian, bladder, salivary gland, endometrial, pancreatic and non-small-cell lung cancer (NSCLC).

Also provided by the present invention is a method for treating cancer characterized by the overexpression of HER2 and in particular selected from the group comprising breast, ovarian, bladder, salivary gland, endometrial, pancreatic and non-small-cell lung cancer (NSCLC).

The present invention also relates to a pharmaceutical formulation for use in the treatment of a patient in need thereof using combinations of the disclosed bispecific antibody and a second immuno-oncology agent to the patient either sequentially or simultaneously.

According to another aspect of the present invention, there is provided a method of treating a patient in need thereof using combinations of the disclosed bispecific antibody and a second immuno-oncology agent to the patient either sequentially or simultaneously.

As used herein, the term "subject" includes any human or nonhuman animal. The term "nonhuman animal" includes all vertebrates, e.g., mammals and non-mammals, such as nonhuman primates, sheep, dogs, cats, horses, cows, chickens, amphibians, reptiles, etc. Preferably the subject is human.

A "patient" for the purposes of the present invention includes both humans and other animals, preferably mammals and most preferably humans. Thus the antibodies of the present invention have both human therapy and veterinary applications. The term "treatment" or "treating" in the present invention is meant to include therapeutic treatment, as well as prophylactic, or suppressive measures for a disease or disorder. Thus, for example, successful administration of an antibody prior to onset of the disease results in treatment of the disease. As another example, successful administration of an antibody after clinical manifestation of the disease to combat the symptoms of the disease comprises treatment of the disease.

"Treatment" and "treating" also encompasses administration of an antibody after the appearance of the disease in order to eradicate the disease. Successful administration of an antibody after onset and after clinical symptoms have developed, with possible abatement of clinical symptoms and perhaps amelioration of the disease, comprises treatment of the disease. Those "in need of treatment" include mammals already having the disease or disorder, as well as those prone to having the disease or disorder, including those in which the disease or disorder is to be prevented.

The antibody or of the present invention can be administered via one or more routes of administration using one or more of a variety of methods known in the art. As will be appreciated by the skilled artisan, the route and/or mode of administration will vary depending upon the desired results. Preferred routes of administration include intravenous, intramuscular, intradermal, intraperitoneal, subcutaneous, spinal or other parenteral routes of administration, for example by injection or infusion. More preferred routes of administration are intravenous or subcutaneous. The phrase "parenteral administration" as used herein means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural and intrasternal injection and infusion. Alternatively, an antibody of the invention can be administered via a non- parenteral route, such as a topical, epidermal or mucosal route of administration, for example, intranasally, orally, vaginally, rectally, sublingually or topically.

The antibody of the present invention can be administered at a single or multiple doses. The term “dose” or “dosage” as used in the present invention are interchangeable and indicates an amount of drug substance administered per body weight of a subject or a total dose administered to a subject irrespective to their body weight.

In accordance with one aspect of the present invention, the pharmaceutical formulations provided may be administered to individuals. Administration is preferably in a "therapeutically effective amount", this being sufficient to show benefit to a subject. Such benefit may be at least amelioration of at least one symptom. The actual amount administered, and rate and time-course of administration, will depend on the nature and severity of what is being treated. Prescription of treatment, e.g. decisions on dosage etc, is within the responsibility of medical doctors. Appropriate doses of antibody are well known in the art (Ledermann JA et al., (1999) Int J Cancer 47: 659-664; Bagshawe KD et a/., (1991) Antibody, Immunoconjugates and Radiopharmaceuticals, 4: 915-922). The precise dose will depend upon a number of factors, including the size and location of the area to be treated, body weight of the subject, the precise nature of the antibody (e.g. whole antibody or fragment) and any additional therapeutic agents administered before, at the time of or after administration of the antibody.

Figure 1: Graphics related to physical-chemical attributes (concentration by UV detection, pH by SevenExcellence pH meter, A) and to size variants (SE-HPLC, B) for BEAT®1 control and stress temperature samples.

Figure 2: Graphics related to purity determination by cGE (NR) for BEAT®1 BDS and FBDS temperature control and stress samples.

Figure 3: Graphics related to purity determination by cGE (R) for BEAT®1 BDS and FBDS temperature control and stress samples.

Figure 4: Graphics related to charge variants determination by CEX for BEAT®1 FBDS and BDS temperature control samples.

Figure 5: Graphics related to size variants determination by SE-HPLC for BEAT®1 FBDS and BDS samples treated with oxidizing agents.

Figure 6: Overlays (left) and Graphs (right) related to charge variants determination by CEX for BEAT®1 BDS and FBDS samples treated with oxidizing agents.

Figure 7: Fit Least Squares reports for SE-HPLC analysis of BEAT®1 BDS (A) and FBDS (B) showing plots of the percentage of aggregates, main and fragments.

Figure 8: Profiler plot with maximized desirability after SE-HPLC analysis (maximum aggregate and fragments levels, minimum main levels) at pH 4 (left) and pH 9 (right) under 40°C temperature incubation for BEAT®1 BDS.

Figure 9: Profiler plot with maximized desirability after SE-HPLC analysis (maximum aggregate and fragments levels, minimum main levels) at pH 4 (left) and pH 9 (right) under 40°C temperature incubation for BEAT®1 FBDS.

Figure 10: Overlays related to purity determination by SE-HPLC for BEAT®1 BDS under pH 4 (A) and pH 9 (B) incubation at 40°C, 10 days

Figure 11: Fit Least Squares reports for cGE (NR) analysis of BEAT®1 BDS (A) and FBDS (B) showing plots of the percentage of BEAT and total fragments.

Figure 12: Overlays related to purity determination by cGE (NR) for BEAT®1 BDS under pH 4 (A) and pH 9 (B) incubation at 40°C, 10 days.

Figure 13: Profiler plot with maximized desirability after cGE (NR) analysis (maximum total fragments levels) at pH 4 (left) and pH 9 (right) under 40°C temperature incubation for BEAT®1 BDS.

Figure 14: Profiler plot with maximized desirability after cGE (NR) analysis (maximum total fragments levels) at pH 4 (left) and pH 9 (right) under 40°C temperature incubation for BEAT®1 FBDS.

Figure 15: Fit Least Squares reports for CEX analysis of BEAT®1 BDS (A) and FBDS (B) showing plots of the percentage of acidics, main and basics.

Figure 16: Profiler plot with maximized desirability after CEX analysis (maximum acidic and basic levels, minimum main levels) at pH 4 (left) and pH 9 (right) under 40°C temperature incubation for BEAT®1 BDS.

Figure 17: Profiler plot with maximized desirability after CEX analysis (maximum acidic and basic levels, minimum main levels) at pH 4 (left) and pH 9 (right) under 40°C temperature incubation for BEAT®1 FBDS.

Figure 18: Graphics related to size variants determination by SE-HPLC for BEAT®1 FBDS and BDS samples under light stress.

Figure 19: Graphics related to purity determination by cGE (NR) for BEAT®1 FBDS and BDS samples under light stress.

Figure 20: Overlays (left) and graphics (right) related to charge variants by CEX for BEAT®1 FBDS and BDS samples under light stress.

Figure 21: Overlays related to purity determination by cGE (R) for BEAT®1 BDS samples under forced glycation (liquid condition)

Figure 22: Overlays related to purity determination by cGE (R) for BEAT®1 BDS samples under forced glycation (lyophilized condition)

Figure 23: Overlays related to purity determination by cGE (NR) for BEAT®1 BDS samples under forced glycation (liquid condition)

Figure 24: Overlays related to purity determination by cGE (NR) for BEAT®1 BDS samples under forced glycation (lyophilized condition).

Example 1: Pre-formulation studies

Pre-formulation studies were performed to assess the effect of histidine buffer concentration, pH and excipient the BEAT®1 bispecific antibody, as well as to assess the effect of lyophilization on different formulations.

First, the effect of pH and excipient content on BEAT®1 formulation was studied before (T0) and after 3 cycles of Freeze Thaw (FT) from -80±20°C to 5±3°C. The sample stability was determined by SE-HPLC (briefly, by employing a linear flow of aqueous buffer at a flow rate of 1 mL/min and an injection volume of 50 µl; separation was achieved by using a TSK gel G3000 column) and by measuring BEAT®1

concentration by absorption at 280 nm using Nanodrop 2000 equipment (Thermofisher) before and after subjecting the sample to the FT cycles; results shown in Table 1.

Histidine Buffer	Man-nitol %	Sucrose %	Glycine %	PS80 %	Arginine %	Glutamate %	PVP %	βCD %	Concentration BEAT®1	Monomer %		OD280	
										T0	FT	T0	FT
5 mM - pH 6	0	0	0.4	0.05	-	-	-	-		96.2	96	0.95	0.96
5 mM - pH 6	2	2	0	0.05	-	-	-	-		96.3	96.1	0.93	0.93
5 mM - pH 6	2	15	0	0.05	-	-	-	-		96.4	96.4	1	1.01
5 mM - pH 6	4	10	0	0.05	-	-	-	-		96.5	96.7	0.98	0.95
5 mM - pH 6	4	15	0	0.05	-	-	-	-		96.1	96.5	0.98	0.96
5 mM - pH 6	6	15	0	0.05	-	-	-	-		96.1	96.1	0.98	0.98
5 mM - pH 6	0	6	0.4	0.05	-	-	-	-		96.4	96.3	0.94	0.96
5 mM - pH 6	0	10	0.8	0.05	-	-	-	-		96.2	96.6	0.93	0.96
5 mM - pH 6	0	15	1.2	0.05	-	-	-	-		96.3	96.4	0.97	0.97
5 mM - pH 6.3	4	15	0	0.05	-	-	-	-		96.4	96.5	0.99	0.96
5 mM - pH 6.3	2	2	0	0.05	-	-	-	-	1	96.2	96.1	0.95	0.96
5 mM - pH 6.3	0	6	0.4	0.05	-	-	-	-		96.1	96.5	0.97	0.94
5 mM - pH 6.3	0	10	0.8	0.05	-	-	-	-		96.1	96.5	1.01	0.95
5 mM - pH 6.5	4	15	0	0.05	-	-	-	-		96.2	96.2	0.9	0.89
5 mM - pH 6.5	2	2	0	0.05	-	-	-	-		96.2	96	0.88	0.86
5 mM - pH 6.5	0	6	0.4	0.05	-	-	-	-		96.3	96.5	0.88	0.88
5 mM - pH 6.5	0	10	0.8	0.05	-	-	-	-		96.2	96.7	0.93	0.72
15 mM - pH 6	4	15	0	0.05	-	-	-	-		96.4	96.7	1.09	1.06
15 mM - pH 6	2	2	0	0.05	-	-	-	-		96.5	96.4	0.93	0.93
15 mM - pH 6	0	6	0.4	0.05	-	-	-	-		96.3	96.6	0.98	0.99
15 mM - pH 6	0	10	0.8	0.05	-	-	-	-		96.5	96.7	0.98	0.94
5 mM - pH 6	2	2	0	0.05	-	-	-	-		96.2	96.3	0.28	0.27
5 mM - pH 6	2	15	0	0.05	-	-	-	-		95.8	96.8	0.32	0.3
5 mM - pH 6	4	10	0	0.05	-	-	-	-		95.7	96.5	0.27	0.27
5 mM - pH 6	4	15	0	0.05	-	-	-	-		95.6	96.7	0.33	0.33
5 mM - pH 6	6	15	0	0.05	-	-	-	-	0.3	95.9	96.8	0.31	0.3
5 mM - pH 6	0	6	0.4	0.05	-	-	-	-		96.6	96.9	0.28	0.28
5 mM - pH 6	0	10	0.8	0.05	-	-	-	-		96.3	96.9	0.3	0.25
5 mM - pH 6	0	15	1.2	0.05	-	-	-	-		95.9	96.2	0.3	0.3
5 mM - pH 6	4	15	0	0.05	0.4	-	-	-		95.4	95.5	1	1
5 mM - pH 6	2	2	0	0.05	0.4	-	-	-		95.9	95.7	0.96	0.91
5 mM - pH 6	0	10	0.8	0.05	0.4	-	-	-		95.8	96	0.95	0.93
5 mM - pH 6	4	15	0	0.05	-	0.4	-	-	1	95.6	96	1.01	0.99
5 mM - pH 6	2	2	0	0.05	-	0.4	-	-		95.7	95.8	0.97	0.97
5 mM - pH 6	0	10	0.8	0.05	-	0.4	-	-		95.7	96	0.99	0.95

5 mM - pH 6	4	15	0	0.05	-	-	0.1	-		96.8	92.8	0.99	0.99
5 mM - pH 6	2	2	0	0.05	-	-	0.1	-		96.6	96.5	0.95	0.94
5 mM - pH 6	0	10	0.8	0.05	-	-	0.1	-		96.8	96.6	0.96	0.99
5 mM - pH 6	4	15	0	0.05	-	-	-	0.1		96.3	96.5	0.99	0.96

Table 1. Effect of pH and excipient. Stress: Freeze Thaw from -80°C to 5°C, 3 cycles.

Based on the results above, formulations containing mannitol and/or sucrose and/or glycine and PS80 and 1 mg/mL and 0.3 mg/mL of BEAT®1 were lyophilized according to the steps of Table 2.

Step	Temperature (°C)	Vacuum (m Torrs)	Ramp Time (h:m)	Hold Time (h:m)
Precooling	5	-	0:20	7:00
Freezing	0	-	0:05	0:30
Freezing	-45	-	0:45	2:00
Freezing	-15	-	0:30	2:00
Freezing	-45	-	0:30	2:00
Vacuum	-45	94	-	-
Primary Drying	-35	75	1:00	120:00
Secondary Drying	25	-	20:00	10:00

Table 2. Lyophilization process

Parameters including cake appearance by visual observation and particle size (radius)by Dynamic light scattering analysis, , reconstitution time, BEAT®1 stability, concentration and pH after reconstruction were assessed for several compositions, results for the 1 mg/mL BEAT®1 and 0.3 mg/mL BEAT®1 containing formulations are shown in Table 3 and Table 4, respectively.

Buffer	Man-nitol %	Sucrose %	Glycine %	PS80 %	Concentration BEAT®1 (mg/mL)	Radius (cummulant fit)		Cake appearance	Reconstitution time (min)	OD 280 (Nanodrop)		Monomer %		pH after Lyo
						T0	Lyo			T0	Lyo	T0	Lyo	
5 mM Histidine pH 6	5	5	0.5	0.06	1	3.6	17.2	Y	1.52	1.007	0.971	98.2	96.7	6.057
	5	10	1	0.01		NA	NA	Y	1.09	NA	NA	NA	NA	NA
	0	10	0	0.11		NA	NA	Y	2.46	NA	NA	NA	NA	NA
	2.5	5	0.5	0.06		4.1	14.6	N	0.58	1.006	0.977	98.2	97.3	6.065
	0	10	1	0.01		4	3.9	Y	0.4	1.039	0.94	98.4	97.7	6.065
	2.5	5	0.5	0.06		3.8	10.7	Y	0.35	1.049	0.957	98	97.4	6.071
	2.5	5	1	0.06		NA	NA	Y	0.23	NA	NA	NA	NA	NA
	0	10	0	0.01		3.8	49.9	Y	0.4	1.044	0.955	98.4	98.2	6.068

	2.5	0	0.5	0.06		4.5	8.1	Y	0.2	0.979	0.988	97.8	93.4	6.078
	2.5	5	0.5	0.11		4.2	10	Y	0.28	1.032	0.989	96.8	96.7	6.08
	5	0	0	0.11		4.4	12.3	Y	0.43	1.006	0.972	97.4	85.8	6.07
	0	5	0.5	0.06		4.3	5.2	Y	0.23	0.984	0.943	98.1	97.6	6.082
	2.5	5	0.5	0.01		4.1	5.5	Y	0.42	0.999	0.958	98.4	97.3	6.081
	5	0	0	0.01		4.5	47.5	Y	0.46	1.018	1.044	97.9	82.3	6.056
	5	0	1	0.11		4.2	10.1	Y	0.5	1.037	0.989	97.5	85.1	6.069
	5	10	0	0.01		NA	NA	N	1.06	NA	NA	NA	NA	NA
	5	10	1	0.11		4.1	10.2	Y	0.39	1.055	0.909	97.9	97	6.056
	2.5	10	0.5	0.06		NA	NA	N	0.44	NA	NA	NA	NA	NA
	0	10	1	0.11		4.1	5.3	Y	0.53	1.063	0.95	97.8	97.4	6.07
	5	10	0	0.11		3.7	37.2	Y	0.55	1.058	1.004	97.9	96.4	6.057
	5	0	1	0.01		4.1	41	Y	0.58	1.002	1.138	98.3	89.1	6.057
	2.5	5	0	0.06		4.2	33	Y	0.27	1.013	0.938	98	97.1	6.062

Table 3. Effect of lyophilization on 1 mg/mL BEAT®1 containing formulations

Buffer	Man-nitol %	Sucrose %	Glycine %	PS80 %	Concentration BEAT®1 (mg/mL)	Radius (cummulant fit)		Cake ap-pearance	Reconstitution (min)	OD 280 (Nanodrop)		Mono-mer %		pH after Lyo
						T0	Lyo			T0	Lyo	T0	Lyo	
5 mM Histidine pH 6	5	5	0.5	0.06	0.3	3.5	34.1	Y	0.35	0.284	0.279	98.4	94.7	NA
	0	0	1	0.11		NA	NA	N	0.22	NA	NA	NA	NA	NA
	5	10	1	0.01		NA	NA	Y	0.55	NA	NA	NA	NA	NA
	0	10	0	0.11		NA	NA	Y	1.07	NA	NA	NA	NA	NA
	2.5	5	0.5	0.06		3.6	17.8	Y	0.24	0.306	0.282	98.2	96.4	NA
	0	10	1	0.01		4	3.8	N	0.26	0.316	0.299	99	98.5	6.087
	2.5	5	0.5	0.06		3.9	16.8	Y	0.36	0.292	0.286	98.2	96.5	NA
	2.5	5	1	0.06		NA	NA	N	0.33	NA	NA	NA	NA	NA
	0	10	0	0.01		4.1	3.6	Y	0.32	0.316	0.297	99	98.1	6.066
	2.5	0	0.5	0.06		3.9	12.5	Y	0.2	0.272	0.295	98.2	85.8	NA
	2.5	5	0.5	0.11		3.7	24.1	Y	0.37	0.302	0.299	96.9	95.7	NA
	5	0	0	0.11		3.5	14.2	Y	0.35	0.307	0.342	97.3	78.4	NA
	0	0	0	0.01		NA	NA	N	0.09	NA	NA	NA	NA	NA
	0	5	0.5	0.06		5	9.5	Y	0.25	0.297	0.299	98.4	97.6	6.09
	2.5	5	0.5	0.01		3.7	4.4	Y	0.33	0.279	0.272	99.1	97.4	6.084
	5	0	0	0.01		3.5	63.5	Y	0.4	0.298	0.384	98.4	67	NA
	5	0	1	0.11		3.3	12.2	Y	0.33	0.313	0.35	97.1	77.5	NA
	0	0	1	0.01		NA	NA	N	0.29	NA	NA	NA	NA	NA
	5	10	0	0.01		NA	NA	Y	0.27	0.373	0.338	99	96.2	NA
	5	10	1	0.11		3.1	12.8	Y	0.46	0.325	0.301	97.1	95.1	NA
	2.5	10	0.5	0.06		NA	NA	Y	1	NA	NA	96.9	NA	NA

	0	10	1	0.11		4.1	9.5	Y	0.31	0.346	0.312	97.6	96.6	6.097
	0	0	0	0.11		NA	NA	N	0.11	NA	NA	NA	NA	NA
	5	10	0	0.11		3.2	40.6	Y	0.26	0.333	0.316	99.1	94.1	NA
	5	0	1	0.01		3.4	81.6	Y	0.48	0.281	0.367	99	80.3	NA
	2.5	5	0	0.06		4.4	46.7	Y	0.33	0.306	0.296	98.1	95.8	NA

Table 4. Effect of lyophilization on 0.3 mg/mL BEAT®1 containing formulations

Based on the results above, formulations F1 to F5 (Table 5) were selected for further optimizing the freezing steps of the lyophilization process (Table 6).

Formulation	Buffer	Mannitol %	Sucrose %	Glycine %	Tween 80 %	Concentration **BEAT®1 g/L**
F1	5 mM Hist pH 6.3	5	5		0.02	0.1
F2	5 mM Hist pH 6.3	2.5	5		0.02	
F3	5 mM Hist pH 6.3	5	5	0.5	0.02	
F4	5 mM Hist pH 6.3	2.5	5	0.5	0.02	
F5	5 mM Hist pH 6.3		5		0.02	

Table 5. Formulations F1 to F5

	Freezing parameters			
Cycle	Step	Temperature (°C)	Ramp Time (hr:min)	Hold Time (hr:min)
1	Precooling	5	00:20	10:30
	Freezing 1	0	00:05	00:30
	Freezing 2	-45	00:45	02:00
	Freezing 3	-15	00:30	02:00
	Freezing 4	-45	00:30	02:00
2	Precooling	5	00:40	10:30
	Freezing 1	0	00:10	00:30
	Freezing 2	-50	01:40	02:00
	Freezing 3	-15	01:10	02:00
	Freezing 4	-50	01:10	02:00
3	Precooling	5	00:10	10:30
	Freezing 1	0	00:02	00:30
	Freezing 2	-45	00:22	02:00
	Freezing 3	-15	00:15	02:00
	Freezing 4	-45	00:15	02:00
4	Precooling	5	00:40	10:30
	Freezing 1	0	00:10	00:30
	Freezing 2	-50	01:40	02:00
	Freezing 3	-25	00:50	06:00
	Freezing 4	-50	00:50	02:00

Table 6. Freezing parameters

		SEC monomer content at different process steps			
Cycle	Formulation	T0	Post Freezing 1	Post Annealing (Freezing 2 and 3)	Post Freezing 3
1	F1	97.9	97.8	97.7	97.6
	F2	97.6	97.7	97.6	97.6
	F3	98	98	97.7	97.8
	F4	97.9	97.7	97.6	97.8
	F5	97.7	97.6	97.7	97.7
2	F1	97.4	NA	NA	97
	F2	97.3	NA	NA	97.6
	F3	97.3	NA	NA	97
	F4	97.2	NA	NA	97.4
	F5	97.1	NA	NA	97.2
3	F1	96.4	95.8	94.1	92.5
	F2	96.5	96.3	95.9	95.9
	F3	95.6	95.8	95.4	94.4
	F4	96.5	95.8	95.9	95.6
	F5	96.8	96.3	96.3	96
4	F1	95.5	95.7	95.6	95.5
	F2	95.3	95.9	95.3	95.4
	F3	96	96.3	96.2	96.1
	F4	95.7	95.7	95.7	95.8
	F5	95.6	95.2	95.3	95.4

Table 7. SEC monomer content results

Based on the result above it was concluded that both formulation composition as well as freezing parameters of the lyophilization process may have an influence on the stability of BEAT®1.

Based on the above experiment, one of the preferred composition for lyophilized presentation comprises of 5 mM histidine, 5% sucrose, 0.02% polysorbate 80 at pH 6.3 have BEAT®1 at a concentration of 0.1 mg/mL.

Example 2: Forced degradation studies

Materials

Forced degradation studies have been carried out on BEAT®1 BDS and FBDS at a concentration of 11.3 mg/mL and 0.1 mg/mL, respectively. FBDS was formulated by diluting the BDS to the desired concentration (0.1 mg/mL) with its formulation buffer: 5 mM L-Histidine, 5% Sucrose, 0.02% Polysorbate 80, pH 6.3, and then filled in 500 mL Freeze-PakTM STS Charter medical bags (FP50ML44) followed by

storage at -80°C before use. For all conditions, 2 mL Fiolax vials (Adelphi Healthcare) were used and filled with 1.1 mL of BEAT®1 BDS (except light stress and freeze-thaw condition) and DP formulation.

Protocol for the different stress conditions

a) T0 unstressed samples

BEAT®1 BDS and FBDS, unstressed samples were directly analyzed at time point T0 upon receiving the material in order to have a first control which will be compared to stressed samples.

b) Temperature stress (5 ± 3°C, 25 ± 2°C and 40 ± 2°C)

The objective of 5 ±3°C and 40 ±2°C temperature conditions was having a control for the forced degraded samples incubated at these temperatures, i.e. samples exposed to acidic and basic pH and forced glycation. Also, 25 ±2°C temperature condition enables having a control for the forced degraded samples incubated at this temperature, i.e. samples exposed to acidic and basic pH, oxidation and light exposure stress. Hence, the goal of the temperature stress is to have a control at various temperatures but also to evaluate the impact of each stress itself on BEAT®1 molecular properties by knowing the influence of the temperature of incubation (Table 1).

c) Oxidation

Oxidation stress condition consisted in oxidizing BEAT®1 BDS and FBDS with AAPH (2,2'-Azobis(2-amidinopropane) dihydrochloride) or H_2O_2 (hydrogen peroxide) which generally oxidizes Tryptophan (Trp) and Methionine (Met) residues respectively. Oxidation by AAPH (Sigma Aldrich) was carried out by mixing protein with AAPH in the final molar concentration of 1:42 (protein:AAPH). A stock solution of AAPH, diluted in highly purified water (HPW) was prepared prior to mixing with BEAT®1 BDS and FBDS. Oxidation by H_2O_2 (Sigma Aldrich) was carried out by mixing protein with H_2O_2 in order to achieve a final concentration of 0.01% H_2O_2. A stock solution was prepared just as AAPH oxidizing agent. The mixed samples with AAPH and H_2O_2 were kept at 25°C and removed at the specific time points (Table 1).

d) Acidic and basic pH

A Design of Experiment (DoE) on JMP statistical software was created in order to characterize, describe, understand and predict multiple factors which influences a resulting quality characteristic. The goals of the study were defining a risk assessment of the X factors, experimental levels and Y responses (analytical methods) was accomplished. The model showed a power analysis above 0.960 for all terms and a variance prediction below 1. The acidic and pH stress condition consisted in adjusting BEAT®1 BDS and FBDS to pH 4, 6.5 and 9 by using minimal quantity of 3.7% HCl and 50% NaOH. Prepared samples were kept

respectively at 5°C, 25°C and 40°C and removed at specific time points for analysis (Table 1). Results were analyzed using multivariate analysis and outliers were identified by Jacknife analysis. Next, all factors and polynomial to degree were added and Standard Least Squares with effect leverage model was constructed.

d) Light exposure stress

Light stress consisted in incubating BEAT®1 BDS and FBDS in a binder light and climatic chamber (Binder). Following conditions have been used for light exposure experiment: 50 mL Freeze-Pak™ STS Charter medical bags (10 mL filling volume), open vessel (50 mL falcon caps with 4 mL filling volume). The selected bag type is the same as the one used for the storage of the BDS material. Bags were lay down in the light exposure chamber. Vials were kept horizontally and caps were placed in a corning plate. Bags/vials/caps were kept on the first shelf of the light chamber, positioned directly below the light source and close together such that the distance from the light source remains similar. As recommended and described in ICH Q1B guidelines (Blessy M. & al, 2014; ICH Q1B), samples were exposed to a minimum of 1.2 mlux hours of visible light and 200 watt-hours of UV-A light. Light intensity was thus measured at each time point with a lux meter (Testo 540)(Table 1).

e) Forced glycation

A 1:1 mass ratio of glucose and a buffer exchange in Phosphate Buffer Saline (PBS) pH 7.0 - 7.5 was used to induce glycation at 37°C. Two different glycations conditions were employed, one in liquid and the other one after lyophilization. The buffer exchange step was done by putting sufficient material (BEAT®1 BDS) into a 15 mL amicon and by fulfilling with PBS pH 7.4. After centrifuging the amicon 15 min at 4000g, the flowthrough was discarded and the washing steps were repeated twice. BEAT®1 BDS was then collected by pipetting. Glycation induction was assessed by adding a 1:1 mass ratio of Alpha-D(+)-Glucose, 99% + %anhydrous (Sigma Aldrich) in the falcon containing the BDS after buffer exchange. The solution was then filtered by using a syringe (Millipore) and a centrifugal filter (Becton Dickenson). Control samples were prepared by adding PBS instead of glucose. For glycation in lyophilized conditions, same steps than liquid glycation induction were followed but after filtration aliquots were prepared in 2 mL Fiolax glass vials (SCHOTT TopLyo, Adelphi Healthcare), stoppered and with a flip-off cap on top. Aliquots were put in the Telstar LyoBeta 15 lyophilizer and lyophilized according to a Glenmark's generic procedure. The same protocol was performed for control samples but prepared with PBS. Liquid glycation samples in sealed tubes (cap with parafilm) and lyophilized glycation samples were incubated at 37°C for 4 months and

aliquots were taken at specific time points (*Table 8*). *Table 8* shows a summary of the different stresses and enzymatic digestions applied to BEAT®1 BDS and FBDS with their respective time points.

Conditions and Time points	T0	pH_DoE*			Oxidation		Light exposure		Forced Glycation	Control 5 ± 3°C, 25 ± 3°C, 40 ± 2°C		
1 day		N/A			X	X	X	X	N/A	N/A	X	N/A
2 days		N/A					X	X	N/A		X	
3 days		X	X	X	X	X	X	N/A	N/A	X	X	X
7 days									X			
10 days	X	X	X	X	X	X	X	X	N/A	X	X	X
2 weeks		N/A							X	N/A		X
1 month									X			X
2 months									X			X
3 months									X			X
4 months									X			X

Table 8: Stability time points for BEAT®1 BDS and FBDS stress conditions. X: Selected time point. N/A: Not Applicable at this time point

Testing at several time points could provide information on the degradation rate and on the degradation products. Once incubation was completed (both time points and stress conditions), aliquots were removed and stored at -80 ± 20°C until analyzed.

Analytical methods

Type of assay	Parameter	Stress / Enzymatic digestion
Appearance*	Appearance	All stresses
Protein Concentration	A280	All stresses
pH measurement	pH value	All stresses
Purity (aggregates)	SE-HPLC	All stresses
Identity (charge variants)	CEX	All stresses
Purity (fragments)	cGE (Reduced)	All stresses
Purity (fragments)	cGE (Non-reduced)	All stresses

Table 9: Summary of the analytical methods performed for BEAT®1 study.* For general appearance, the degree of opalescence (clear, opalescent), the coloration (colorless, Brownish, Yellowish) and the particulates were observed.

a) Total protein determination

Protein concentration enables to determine the quantity of proteins in a solution which absorb ultraviolet light at 280 nm wavelength (A280), due to the presence of aromatic amino acids in the protein structure,

mainly tryptophan and tyrosine. Depending on the stress condition, the concentration value could increase (may also be due to evaporation of the water) or decrease and the material could be impacted. For precise measurement, the concentration of the sample must be 0.1-10.0 mg/mL. Otherwise, if sample concentration is >10.0 mg/mL, which is our case with BEAT®1 BDS, a dilution with the corresponding sample buffer is required. For all BDS samples, the same dilution (2X) was used. Protein concentration by UV-Visible spectrophotometer is determined using Nanodrop 2000 equipment (Thermo scientific).

b) pH measurement

The pH is characterized in order to control the stability of our protein in solution. A change in the pH value could lead to degradation in proteins. With the time, the pH value could change due to the evaporation of the water and the concentration of the excipients of the buffer. Before taking a measurement, calibration of the pH meter is necessary. Minimum 300 µl of solution is required to measure the pH with the nano probe. pH measurements are determined using SevenExcellence pH meter (Mettler Toledo).

c) Size Exclusion-High Performance Liquid Chromatography (SE-HPLC)

Size Exclusion-HPLC allows to determine purity of BEAT®1 in purified samples. The SE-HPLC permits to obtain a percentage value of monomer, aggregate and fragment but is mainly interesting for the percentage of aggregates. A decrease in the percentage value of monomer could mean that the main species is degraded or form either aggregates or fragments. A column with eluent A (0.1M sodium phosphate, 0.15M NaCl, pH 6.8) was used for chromatographic separation (protein separated according to their molecular weight) and at a flow rate of 1.0 mL/min. Samples were not diluted for BEAT®1 FBDS and diluted to 0.3 mg/mL in eluant A for BDS. 50 uL of sample was injected for the chromatographic system. Samples were analyzed using Alliance HPLC system (Waters) and with UV absorbance detection at 280 nm with a Tosoh TSKgel G3000SWXL, 7.8 mm x 30 cm column, 5 um particle size.

d) Cation exchange High Performance Liquid Chromatography (CEX)

CEX-HPLC is used to investigate the identity of BEAT®1. The method allows the determination of the charge profile (positive and negative charges) of BEAT®1 purified samples. If the product is degraded, chemical changes in the molecule could appear and lead to changes in percentages of peak area. A step gradient using eluant A (20 mM MES, pH 6.9) and eluant B (20 mM MES, 150 mM NaCl, pH 7.0) at 1.0 mg/mL was applied. The different forms of antibodies in solution are separated according to their interactions with a cation-exchange column and analyzed after passing through a UV-detector (absorbance at 280 nm). CEX was performed to monitor BEAT®1 charge variants using a ProPac WCX-10 column (4.0 x 250 mm; Dionex).

e) Capillary Gel Electrophoresis (cGE Reduced and Non-reduced)

cGE is used to determine the purity of monoclonal antibodies in terms of fragments and main species. Non-reduced conditions allows to detect fragments and main species (BEAT) of BEAT®1. Reduced conditions allows to detect LC, HC and scFv-Fc species of BEAT®1. The percentage value of main species could decrease if the main species is degraded. Samples were separated by electrophoresis through a sieving gel matrix (Beckman Coulter Kit) in a 50 um I.D. bare-fused silica capillary with 57 cm effective length using a Beckman Coulter PA 800 system with DAD/PDA detector (Diode Array/Photodiode Array Detector).

Results Forced Degradation Studies

Thermal stress (5 ±3°C, 25 ±2°C and 40 ±2°C)

Based on the results from Figure 1 (graphic A), for all temperatures the physical (appearance) was not impacted as well as some of the physico-chemical attributes (pH) of BEAT®1 FBDS and BDS. Unpredictable results were observed concerning the protein concentration of the BDS. Indeed, for all temperatures between day 1 and day 10, the concentration increased by 16 to 20% (11.3 mg/mL to 13.6 mg/mL) compared to T0 unstressed samples which may be due to evaporation for 40°C temperature samples but for 5°C and 25°C control samples.

In terms of size variants (Figure 1, graphic B), FBDS and BDS at 5°C were not impacted and shows that BEAT®1 is relatively stable up to day 10. Interesting results could be observed at 25°C, showing a continuous decrease of the percentage of aggregates from day 1 (-52.3% for FBDS and -27.1% for BDS) to day 3 followed by an increase at day 10 (-37.8% for FBDS and -29.0% for BDS). At 40°C for BEAT®1 shows a slight decrease of the percentage of aggregates from day 3 to day 10 for BDS samples (4.15% to 3.66%) but an increase at day 10 for FBDS samples (0.76% to 2.01%). No relevant variations were shown concerning the variation of the percentage of fragments.

Finally, 40°C temperature is a stress factor as we can suppose that after 1 day reversible aggregates are decreased followed by an increase in the percentage of aggregates when incubation is longer. Moreover, when incubation at high temperature is extended just as a stress factor, this induces aggregates. BDS and FBDS have similar trends, and compared to T0 unstressed samples for all temperatures at either time point, the level of aggregates decrease. The FBDS with a lower concentration, shows a lower level of aggregates. To conclude, this study highlighted that high temperatures have a positive effect on the formation of aggregates.

cGE (NR) analysis in terms of purity (Figure 2) did not show major changes for all temperatures. Slight increase could be observed at 40°C with the percentage of 80 kDa and 100 kDa fragments after 10 days. No significant changes could be notified concerning the percentage of BEAT'+ BEAT compared to T0 unstressed and reference standard (TRS) samples.

For cGE (R) analysis (Figure 3), no major variations were observed for all temperatures between time points concerning the percentage of LC, HC aglycosylated, HC and scFv-Fc. A decrease was noticed when comparing the results with T0 unstressed and TRS samples. This could be due to the incubation of BEAT®1 FBDS and BDS at the respective temperatures (5 ±3°C, 25 ±2°C and 40 ±2°C) which were done at a different time interval and analysis with different equipment. To conclude no major changes could be observed for cGE (NR & R) analysis.

Major changes occurred by CEX analysis in terms of charged variants (Figure 4), showing a slight increase in the percentage of acidic starting at 25°C control temperature (24.5% to 25.9% for FBDS and 21.7% to 22.6% for BDS) and significantly increasing at 40°C (up to 42.9% for FBDS and 29.4% for BDS compared to T0 unstressed samples at day 10). This trend is followed by a slight decrease in the percentage of main at all temperatures while basic variants significantly decreased until day 10 (-4.1% for FBDS and -14.4% for BDS) which could indicate that the main and basic species are degrading to generate acidic species of BEAT®1 upon 25°C and 40°C especially.

Oxidation

Oxidation of methionine and tryptophan are known degradation pathways for monoclonal antibodies. The induced chemical modifications may impact the biological activity of antibodies and may have biological consequences (Andrea Bertolotti-Ciarlat, & al, 2009). These amino acids are also accompanied by other degradants such as aggregates. Thus, BEAT®1 BDS and FBDS samples were induced with H_2O_2 or AAPH.

AAPH and H_2O_2 oxidizing agents did not impact the physical (appearance) attributes of the BDS and FBDS. As shown in Figure 5, AAPH let to more degradation compared to H_2O_2 for BDS in terms of size variants. Aggregates increased mostly and significantly between day 3 and day 10 (1.2% to 33.8% compared to T0) while a slight variation was observed at 25°C (control). As a consequence, this increasing trend could be attributed to oxidation by AAPH and not to the temperature of incubation. Under H_2O_2 oxidation, aggregates decreased from day 3 and increased at day 10 (reversible aggregates). The same trend was observed for the control 25°C with slightly lower change, hence this variation could be attributed to the temperature of incubation. Fragmentation also slightly increased from day 10 for both oxidizing agents

with a higher percentage value of fragments generated with AAPH but the variation is not significant compared to T0 unstressed samples. The method holds some limitation caused by reversible aggregates, phenomena observed in the temperature stress section.

Regarding CEX analysis, we can observe through the graphics and overlays (Figure 6) a significant increase in acidic species between day 1 and day 10, ranging from 4.8% to 21.9% (compared to T0) upon AAPH oxidation for BDS samples. The percentage of main on the opposite, decreased significantly from day 3 and up to -30.9%. Following the same trend, the percentage of basics significantly decreased from day 3 to day 10, ranging from -13% to -29.2%. These trends vary from 25°C control temperature species which suggest that BDS and FBDS samples are impacted under AAPH oxidation. AAPH results in oxidation on the exposed Trp on the LC sequence (Miriam Hensel & al, 2011) which could explain the results obtained with BEAT®1.

Upon H_2O_2 oxidation, we can notice a continuous decrease in terms of acidic species (up to -14%) and main variants up to -12.8% until day 10 for BDS. The decrease is much more significant on the overlays as we can observe a change in the proportion of charge profile and the loss of proteins at the same time. On the other hand, the oxidizing agent significantly generated basic species which are well seen for FBDS and BDS samples. Different trends were observed at 25°C which puts forward the effect of H_2O_2 oxidation. Knowing that this agent generates Met oxidation, it may be possible that exposed residues on BEAT®1 such as on the HC and the ScFv-Fc follow oxidation (Jingjie Mo & al, 2016). Oxidation is known to influence potency/binding activity of monoclonal antibodies (Xuan Gao & al, 2014), thus it is important to analyze the bioactivity of the molecule after oxidation for BEAT®1. In order to determine the specific sites of Met and Trp oxidation and gain knowledge on their location and various covalent modifications, it is proposed to send selected samples for mass spectrometry (MS)-based methods analysis.

Acidic and basic pH

pH treatment at pH 4 enables to investigate the aggregation and fragmentation mechanisms and the stability characteristics of proteins in acidic conditions. The aggregation propensity increases with low pH at a fixed temperature or with increasing temperature at a fixed pH (Erinc Sahin & al, 2011). pH 9 has been tested in order to also follow aggregation and fragmentation mechanisms depending on the temperature applied and to determine the deamidation susceptibility of asparagine residues located at different sites. In order to gain understanding of factors influencing degradation of BEAT®1, their impact, and propose working ranges to gain specific data, DoE experiment was proposed and executed.

Regarding SE-HPLC analysis, the results of the model showed R^2 data for the percentage of aggregates, main and fragments which are above 0.62 for FBDS and BDS (Figure 7). DoE models have identified the pH, temperature and time as significant factors influencing the levels of aggregates, main and fragments. For instance, through the profiler plot (Figure 8), we can observe at pH 4, 40°C and after 10 days a significant increase in the level of aggregates (16.69%) for BDS and a lower percentage change for FBDS (Figure 9). This increase is likely due to combined high temperature and low pH incubation. On the other hand, at pH 9 we can observe a significant rise in the level of fragments which is shown when temperature and time is also increasing (ranging from 0.94% at 5°C to 3.50% at 40°C for FBDS and from 0.65% to 2.82 for BDS at day 10). For both pH values, an increase in the level of aggregates and fragments is correlated to a decrease in main species. This was directly observed on the chromatograms (Figure 10) based on the experimental data which is the basis that led to the creation of the profiler plot adding prediction information.

Moreover, we can observe on the overlay at pH 9, just after the BEAT peak, a shoulder corresponding to a start of fragmentation. We can conclude that high temperature incubation induces significant levels of aggregates and fragments depending on the pH.

The desirability shown in the graphics demonstrate the most appropriate model for both pH and temperature incubation factors such as a worst case scenario. Regarding cGE (NR) analysis, R^2 values are above 0.73 for BDS and 0.63 for FBDS in all cases (Figure 11). We can observe through the overlays (Figure 12) and profiler plot (Figure 13) that the levels of total fragments and BEAT are mainly influenced by high temperature incubation and secondarily by pH value. These data support observations made from SE-HPLC analysis (overlays and graphics). At pH 9, the levels of fragments are higher compared to pH 4 and increased significantly when the temperature rises to 40°C, ranging from 4.97% to 9.30% for BDS and 3.85% to 7.48% for FBDS (Figure 14). As expected, for pH 9 we can notice a percentage of BEAT (90.56%), which is slightly lower compared to pH 9 (92.87%) and thus leads to a significant increase in total fragments. As shown in the overlay at pH 9, the temperature of incubation has a big influence in the appearance of various fragments.

Regarding CEX analysis, the prediction of the model shows a R^2 ranging from 0.70 (% acidics) to 0.59 (% basics) for BDS and 0.78 to 0.75 for FBDS (Figure 15). The levels of variant species are significantly influenced by pH incubation and less by time and temperature incubation. At day 10 under pH 4 incubation (Figure 16), we can observe a low level of acidics (29.05%) which rises significantly at pH 9 (62.61%) and 40°C incubation for BDS (FBDS show slightly lower changes, Figure 17). Inversely, the level

of basics showed maximum percentage at pH 4 (52.74%) compared to pH 9 (25.30%) for BDS, followed by a decrease of main species. The level of basic species for FBDS observed at pH 9 was lower (20.30%). As a consequence, the main species mainly degraded into basic species of BEAT®1 upon low pH and acidic species upon high pH and was secondarily influenced by the temperature. Elevated pH condition may have induced specific deamidation on the HC and the ScFv-Fc sequence of BEAT®1 (presence of Asn-Gly, Asn-Ser, Asn-Thr, Asn-Asn) which could be verified by MS analysis (Lihua & al, 2005).

By extracting data with the profiler plot generated for SE-HPLC analysis, we can notice that the optimal pH for BDS is between 6.0 and 7.0, which is in range of the formulation solution pH (6.3). Another observation in relation to pH is the possibility to detect chemical degradations that could decrease bioactivity and induce toxicity. In order to have precise data on impacted amino acids of BEAT®1, MS analysis is required. This will enable to know which amino acids are the most exposed and susceptible to degradation.

Light exposure

Light exposure causes a combination of physical and chemical degradation reactions towards antibodies including covalent aggregate formation, fragmentation at the hinge region, oxidation of Trp, Histidine and Met residues and deamidation of Asparagine residues (Pei Qi & al, 2008). In this study BEAT®1 BDS has been filled in BDS bags and in open vessels (50 mL Falcon caps) and FBDS in stoppered glass vials. BEAT®1 formulations have been exposed to light as described in ICH guidelines. Light stress did not impact the physical (appearance) and physico-chemical attributes (protein concentration and pH) of the BDS and FBDS.

In terms of size variants (Figure 18), we can observe significant percentage of aggregates for FBDS and BDS samples. For BDS samples, aggregates decrease and increase between day 1 and day 10, thus showing reversible aggregates. These trends were also observed at 25°C (control) for both kind of samples. As a consequence, this variation could be attributed to the temperature.

On the contrary, percentage of fragments increased significantly up to 833.3% for FBDS and 82.4% for (compared to T0) between day 1 and day 10 compared to 25°C with much lower change, suggesting that light stress has an impact on fragments of BEAT®1.

cGE (NR) analysis (Figure 19) confirms results obtained with SE-HPLC. Indeed fragments increased significantly and shows a percentage LC ranging up to 140% for FBDS and 125% for BDS at day 10. The same trend was observed for the percentage 100 kDa which shows a continuous significant increase up

to 116.7% for FBDS and 73.7% for BDS until day 10. Under 25°C control temperature trends were more constant, therefore, this increase could be attributed to the stress itself. The increase in both LC and 100 kDa species could then explain the increase in the percentage of fragments observed in SE-HPLC.

Regarding CEX analysis in terms of charge variants Figure 20), it is well observed that the percentage of acidic significantly increased for both formulations (up to 94.9% for FBDS and 99.4% for BDS) until day 10, trend also observed at 25°C but with much lower percentage of change. Thus, this increasing trend could mainly be attributed to the stress and secondarily to the temperature.

The percentage of main and basics on the other hand significantly decreased which suggests that these variants are degraded upon light stress to generate acidic variants. On the other hand, we can notice the disappearance of an acidic peak with retention time around 9.5 minutes after 10 day of incubation for both products. It could be intriguing to collect the specific peak from stressed samples to have more characterization prior MS analysis.

It is interesting to notice that there is a correlation of the species detected between CEX analysis upon AAPH oxidation and light stress. The trends are similar for light stress suggesting that oxidation peaks could be observed, thus Trp oxidation or other amino acids (Tyr, Phe, His, Cys) may be induced by light stress (Pei Qi & al, 2008). MS analysis would help to get more information about the impact on BEAT®1 amino acid sequences.

Forced Glycation

Glycation is an important protein modification that could potentially affect bioactivity and molecular stability.

Upon liquid glycation, we can observe through cGE (R) analysis and overlays (Figure 21, overlays B) that the glycation mainly impacted LC of BEAT®1. One shoulder on the LC can be distinguished from week 1 and increased until week 8. While the shoulder is increasing, the main peak of the LC is decreasing. This indicates that the shoulder of the LC correspond to the LC with glycation. The more glycation is observed, the bigger the shoulder gets. Regarding HC and ScFv-Fc, we can observe a decrease from week 2 to week 4 and that the peaks are getting broader. On top of that, HC and ScFv-Fc are slightly shifting to the right from week 8, showing a progressive mass shift. The following time points at week 12 and 16 should show more pronounced changes, hence the beginning of an overlapping of HC and ScFv-Fc and a significant increase in the formation of a shoulder on the LC.

Upon glycation in lyophilized condition, we can observe through cGE (R) analysis and overlays Figure 22, overlays B) that the glycation impacted all peaks such as LC, HC and ScFv-Fc. First of all, the main peak of the LC decreased continuously from week 1 until week 8. From week 1 to week 2, 2 shoulders can be observed compared to liquid glycation where only one shoulder was noted. But from week 4 to week 8, it is hard to distinguish the LC from the glycated LC which completely modified. At week 4, little bumps are noticeable on the LC peak. It will be interesting to look at the next time points in order to see the evolution of the LC peak from week 8.

We can enumerate that from our last time point, the peak shows total LC plus glycated LC. Compared to liquid glycation, glycation seems to occur a lot quicker for the lyophilized condition and also leads to more glycation.

Next, the HC peak is strongly affected by glycation in lyophilized condition as compared to liquid condition because all along the glycation kinetic a mass shift to the right can be observed and finally from week 2 both HC and ScFv-Fc peaks couldn't be differentiated and are completely overlapping. We can then notice that the addition of glucose on HC part of the molecule leads to an increase in molecular weight from week 4. In the end, glycated HC and ScFv-Fc show the same molecular weight which is observed on the overlays by one wide peak instead of two sharp peaks. Results for cGE (R) analysis are shown in appendix 2, Figure 23 and Figure 24.

To conclude, after 2 months of glycation in lyophilized and liquid condition all peaks have been impacted (more pronounced for lyophilized condition) for BEAT®1 BDS. Furthermore, the shoulders which were observed on the LC seems to correlate to LC with one or multiple glycation. In order to identify the specific sites of glycation of lysine residues, selected samples will be analyzed by MS.

Example 3: Stability studies – BDS

Next, the stability of BEAT®1 BDS (target BEAT®1 concentration 10±2 mg/mL 5 mM Histidine, 5% w/v Sucrose, and 0.02% w/v Polysorbate 80 at solution pH of 6.3, filled into 50 mL bags (Freeze-Pak™ STS 50 mL, Product code: FP50ML44, Charter Medical) with a fill volume of 15 mL per bag, was studied in different conditions and on including: storage for up to 48 months (48 M) at < -60 °C (Table 10); storage for up to 48 months (48 M) at -20±5°C (Table 11); storage for up to 1 month at + 5±3°C (Table 12 and Table 19); storage for up to 1 month at 25±3°C (Table 13 and Table 20); 3 cycles of FT from -60°C or -20±5°C to 5°C (Table 14); temperature excursions (Table 15); 3 cycles of Freeze-Thaw at both -80 ± 20°C and -20 ± 5°C (Table 16); storage for up to 36 months at -80 ± 20°C (Table 17); storage for up to 36 months at -20 ±

5°C (Table 18); storage at -80°C for 2 weeks or 24M followed by storage for 1 month at + 5 ± 3°C (Table 21); storage at-20°C for 2 weeks or 24 months followed storage for 1 month at + 5 ± 3°C (Table 22); storage for up to 35 months at -80 ± 20°C (Table 23); storage for up to 35 months at -20 ± 5°C (Table 24).

BEAT®1 BDS stability was assessed by comparing the obtained results to reference standards. The measured parameters included pH measurement, physical appearance of the liquid, A280 measurement, SDS-PAGE, 5 SE-HPLC, cGE, CEX-HPLC, HIC-HPLC, cIEF (iCE3), Binding ELISA, functional assay, SDS-PAGE and bioburden analysis.

Test	Specifications	Time Interval (months)										
		T0	T1	T2	T3	T6	T9	T12	T18	T24	T36	T48
Physical appearance of liquid	Colorless to slightly yellowish, clear to slightly opalescent liquid	Clear and Colorless	Clear and Colorless	Clear and Colorless	Clear and Colorless	Clear and Colorless	Clear and Colorless	Clear and Colorless	Clear and Colorless	Slightly Opalescent, Slightly yellowish	Clear and Colorless	Clear and Colorless
pH	T0 ± 0.1 (≥ 6.2 and ≤ 6.4)	6.3	6.3	6.3	6.3	6.2	6.3	6.2	6.2	6.2	6.2	6.2
A280 (mg/mL)	T0 ± 20% (≥ 9.1 mg/mL and ≤ 13.7 mg/mL)	11.4 mg/mL	11.3 mg/mL	11.0 mg/mL	11.4 mg/mL	11.5 mg/mL	11.1 mg/mL	11.2 mg/mL	11.2 mg/mL	11.2 mg/mL	11.2 mg/mL	11.3 mg/mL
SE-HPLC	%Main monomer peak ≥ 95%	98%	98%	98%	98%	97%	98%	98%	97%	95%	98%	98%
CGE (NR)	Comparable to RS	Con-form	Con-form	Con-form	Con-form	Con-form	Con-form	Con-form	Con-form	Conform	Con-form	Con-form
	%BEAT: ≥ 90.0%	96.7%	96.7%	96.6%	96.8%	92.5%	96.8%	95.3%	96.5%	95.7%	96.2%	95.1%
CGE (R)	Comparable to RS	NA	NA	NA	NA	NA	Con-form	Con-form	Con-form	Conform	Con-form	Con-form
	Report %LC						17.1%	20.1%	20.0%	20.0%	20.5%	20.7%
	Report %HC						36.2%	35.4%	35.0%	35.5%	33.7%	33.1%
	Report %ScFv						46.7%	44.2%	44.5%	44.1%	45.5%	46.1%

CEX-HPLC	Comparable to RS	Con-form	Con-form	Con-form	Con-form	Con-form	Con-form	Con-form	Con-form	Conform	Con-form	Con-form
	Report % Acidic peaks	22.1%	20.9%	22.0%	20.9%	23.6%	22.6%	24.8%	23.5%	23.3%	23.0%	22.3%
	Report % Main peak	25.4%	27.4%	24.9%	26.7%	26.3%	27.3%	27.9%	25.7%	25.6%	26.8%	25.8%
	Report % Basic peaks	52.5%	51.6%	53.1%	52.4%	50.1%	50.1%	47.4%	50.8%	51.1%	50.2%	51.9%
HIC-HPLC	Comparable to RS	Con-form	NA	NA	NA	NA	NA	NA	Con-form	Conform	Con-form	Con-form
	Report % BEAT	97.9%							99.5%	99.7%	99.6%	99.5%
cIEF (iCE3)	Comparable to RS	Con-form	Con-form	Con-form	Con-form	Con-form	Con-form	Con-form	Con-form	Conform	Con-form	Con-form
	Report %Acidic species	22.6%	23.7%	23.4%	23.8%	23.7%	22.5%	23.0%	23.1%	22.8%	25.5%	22.5%
	Report %Main specie	40.8%	40.8%	41.0%	39.9%	43.2%	41.9%	43.2%	41.3%	43.1%	41.3%	40.9%
	Report %Basic species	36.6%	35.5%	35.6%	36.3%	33.1%	35.5%	33.8%	35.6%	34.1%	33.2%	36.6%
Binding ELISA	≥ 50% and ≤ 150% of EC50 of Ref. Std (RS).	100%	102%	96%	94%	77%	75%	126%	84%	96%	100%	91%
Functional Assay (g)	≥ 50% and ≤ 150% of EC50 of Ref. Std.	87%	NA	NA	147%	91%	99%	132%	109%	112%	84%	91%
SDS-PAGE (Reduced)	Main bands approx. 25kDa and 50kDa; Banding pattern consistent with reference standard.	Con-form	Con-form	Con-form	Con-form	NA	Con-form	Con-form	NA	Conform	Con-form	Con-form

SDS-PAGE (Non-reduced)	Main band approx. 125kDa; Banding pattern consistent with reference standard.	Con-form	Con-form	Con-form	Con-form	NA	Con-form	Con-form	NA	Conform	Con-form	Con-form

Table 10. Stability at < -60 °C up to 48 months of Lot N.1

Test	Specifications	Time Interval (months)										
		T0	T1	T2	T3	T6	T9	T12	T18	T24	T36	T48
Physical appearance of liquid	Colorless to slightly yellowish, clear to slightly opalescent liquid	Clear and Colorless	Clear and Colorless	Clear and Colorless	Clear and Colorless	Clear and Colorless	Clear and Colorless	Clear and Colorless	Clear and Colorless	Slightly Opalescent, Slightly yellowish	Clear and Colorless	Clear and Colorless
pH	T0 ± 0.1 (≥ 6.2 and ≤ 6.4)	6.3	6.3	6.3	6.3	6.2	6.3	6.2	6.2	6.2	6.2	6.2
A280 (mg/mL)	T0 ± 20% (≥ 9.1 mg/mL and ≤ 13.7 mg/mL)	11.4 mg/mL	11.3 mg/mL	11 mg/mL	11.5 mg/mL	11.4 mg/mL	11.1 mg/mL	11.5 mg/mL	11.0 mg/mL	11.0 mg/mL	11.3 mg/mL	11.5 mg/mL
SE-HPLC	%Main monomer peak ≥ 95%	98%	98%	98%	98%	97%	97%	98%	97%	95%	98%	98%
CGE (NR)	Comparable to RS	Con-form	Con-form	Con-form	Con-form	Con-form	Con-form	Con-form	Con-form	Con-form	Con-form	Con-form
	%BEAT: ≥ 90.0%	96.7%	97.0%	96.6%	96.9%	90.0%	96.8%	96.0%	96.4%	95.8%	96.5%	95.0%
CGE (R)	Comparable to RS	NA	NA	NA	NA	NA	Con-form	Con-form	Con-form	Con-form	Con-form	Con-form
	Report %LC						17.0%	20.2%	19.9%	19.9%	20.2%	20.8%
	Report %HC						35.4%	35.4%	34.9%	35.4%	33.5%	32.8%
	Report %ScFv						47.6%	44.0%	44.8%	44.1%	46.1%	46.3%

CEX-HPLC	Comparable to RS	Con-form	Con-form	Con-form	Con-form	Con-form	Con-form	Con-form	Con-form	Con-form	Con-form	Con-form
	Report % Acidic peaks	22.1%	21.0%	22.0%	21.2%	23.5%	22.9%	23.7%	23.8%	23.3%	22.5%	22.6%
	Report % Main peak	25.4%	27.4%	24.4%	26.8%	26.4%	27.3%	28.9%	25.4%	25.5%	26.9%	25.5%
	Report % Basic peaks	52.5%	51.6%	53.7%	52.1%	50.1%	49.7%	47.4%	50.8%	51.2%	50.5%	51.9%
HIC-HPLC	Comparable to RS	Con-form	NA	NA	NA	NA	NA	NA	Con-form	Con-form	Con-form	Con-form
	Report % BEAT	97.9%							99.5%	99.7%	99.6%	99.5%
cIEF (iCE3)	Comparable to RS	Con-form	Con-form	Con-form	Con-form	Con-form	Con-form	Con-form	Con-form	Con-form	Con-form	Con-form
	Report %Acidic species	22.6%	24.2%	23.7%	23.9%	24.5%	22.9%	22.9%,	23.4%	22.8%	25.7%	22.8%
	Report % Main specie	40.8%	40.4%	40.5%	40.1%	46.2%	41.3%	43.4%	41.0%	43.4%	41.3%	40.7%
	Report %Basic species	36.6%	35.3%	35.8%	36.0%	29.2%	35.8%	33.7%	35.7%	33.8%	33.0%	36.5%
Binding ELISA	≥ 50% and ≤ 150% of EC50 of Ref. Std (RS).	100%	106%	112%	107%	93%	120%	119%	105%	90%	89%	104%
Functional	≥ 50% and ≤ 150% of EC50 of Ref. Std.	87%	NA	NA	75%	125%	81%	101%	91%	72%	100%	93%
SDS-PAGE (Reduced)	Main bands approx. 25kDa and 50kDa; Banding pattern consistent with reference standard.	Con-form	Con-form	Con-form	Con-form	NA	Con-form	Con-form	NA	Con-form	Con-form	Con-form

SDS-PAGE (Non-reduced)	Main band approx. 125kDa; Banding pattern consistent with reference standard.	Con-form	Con-form	Con-form	Con-form	NA	Con-form	Con-form	NA	Con-form	Con-form	Con-form

Table 11. Stability at -20±5°C up to 48 months of Lot N.1

Stability Indicating Tests	**Specifications**	**Time Interval (months)**		
		Initial T0	**T0.5**	**T1**
Physical appearance of liquid	Colourless to slightly yellowish, clear to slightly opalescent liquid.	Clear and Colorless	Clear and Colorless	Clear and Colorless
pH	T0 ± 0.1 (≥ 6.2 and ≤ 6.4)	6.3	6.2	6.3
A280	T0 ± 20% (≥ 9.1 mg/mL and ≤ 13.7 mg/mL)	11.4 mg/mL	11.2 mg/mL	11.5 mg/mL
SE-HPLC	% Main monomer peak ≥ 95%	98%	98%	98%
CGE (NR)	Comparable to RS	Conform	Conform	Conform
	% BEAT: ≥ 90.0%	96.7%	96.2%	97.1%
CEX-HPLC	Comparable to RS	Conform	Conform	Conform
	Report % Acidic peaks	22.1%	23.0%	22.1%
	Report % Main peak	25.4%	25.8%	27.4%
	Report % Basic peaks	52.5%	51.2%	50.6%
cIEF (iCE3)	Comparable to RS	Conform	Conform	Conform
	Report %Acidic species	22.6%,	23.7%,	25.0%,
	Report %Main specie	40.8%,	41.7%,	40.4%,
	Report %Basic species	36.6%	34.7%	34.6%
Binding ELISA	≥ 50% and ≤ 150% of EC50 of Ref. Std.	100%	98%	127%
Functional Assay	≥ 50% and ≤ 150% of EC50 of Ref. Std.	87%	NA	NA
SDS-PAGE (Reduced)	Main bands approx. 25kDa and 50kDa; Banding pattern consistent with reference standard	Conform	Conform	Conform
SDS-PAGE (Non-reduced)	Main band approx. 125kDa; Banding pattern consistent with reference standard.	Conform	Conform	Conform

Table 12. Stability at + 5±3°C up to 1 month of Lot N.1

Stability Indicating Tests	Specifications	Time Interval (months)		
		Initial T0	T0.5	T1
Physical appearance of liquid	Colourless to slightly yellowish, clear to slightly opalescent liquid.	Clear and Colorless	Clear and Colorless	Clear and Colorless
pH	T0 ± 0.1 (≥ 6.2 and ≤ 6.4)	6.3	6.2	6.3
A280	T0 ± 20% (≥ 9.1 mg/mL and ≤ 13.7 g/L)	11.4 mg/mL	11.9 mg/mL	12.2 mg/mL
SE-HPLC	% Main monomer peak ≥ 95%	98%	98%	98%
CGE (NR)	Comparable to RS	Conform	Conform	Conform
	% BEAT: ≥ 90.0%	96.7%	96.5%	96.6%
CEX-HPLC	Comparable to RS	Conform	Conform	Conform
	Report % Acidic peaks	22.1%	24.0%	25.6%
	Report % Main peak	25.4%	24.4%	24.3%
	Report % Basic peaks	52.5%	50.6%	50.2%
cIEF (iCE3)	Comparable to RS	Conform	Conform	Conform
	Report % Acidic peaks	22.6%	25.9%	29.5%
	Report % Main peak	40.8%	40.7%	38.3%
	Report % Basic peaks	36.6%	33.4%	32.2%
Binding ELISA	≥ 50% and ≤ 150% of EC50 of Ref. Std.	100%	100%	108%
Functional Assay	≥ 50% and ≤ 150% of EC50 of Ref. Std.	87%	NA	NA
SDS-PAGE (Reduced)	Main bands approx. 25kDa and 50kDa; Banding pattern consistent with reference standard	Conform	Conform	Conform
SDS-PAGE (Non-reduced)	Main band approx. 125kDa; Banding pattern consistent with reference standard.	Conform	Conform	Conform

Table 13. Stability at +25±2°C up to 1 month of Lot N.1

Stability Indicating Tests	Specifications	Time Interval (months)		
		Initial T0	3FT (< -60°C)	3FT (-20 ± 5°C)
Physical appearance of liquid	Colourless to slightly yellowish, clear to slightly opalescent liquid.	Clear and Colorless	Clear and Colorless	Clear and Colorless
pH	T0 ± 0.1 (≥ 6.2 and ≤ 6.4)	6.3	6.2	6.2
A280	T0 ± 20% (≥ 9.1 mg/mL and ≤ 13.7 g/L)	11.4 mg/mL	11.2 mg/mL	11.3 mg/mL

SE-HPLC	% Main monomer peak ≥ 95%	98%	98%	97%
CGE (NR)	Comparable to RS	Conform	Conform	Conform
	% BEAT: ≥ 90.0%	96.7%	96.9%	97.0%
CEX-HPLC	Comparable to RS	Conform	Conform	Conform
	Report % Acidic peaks	22.1%	22.3%	22.5%
	Report % Main peak	25.4%	26.3%	26.0%
	Report % Basic peaks	52.5%	51.5%	51.5%
cIEF (iCE3)	Comparable to RS	Conform	Conform	Conform
	Report % Acidic peaks	22.6%	22.3%	23.0%
	Report % Main peak	40.8%	42.6%	42.4%
	Report % Basic peaks	36.6%	35.1%	34.6%
Binding ELISA	≥ 50% and ≤ 150% of EC50 of Ref. Std.	100%	103%	85%
Functional Assay	≥ 50% and ≤ 150% of EC50 of Ref. Std.	87%	67%	69%
SDS-PAGE (Reduced)	Main bands approx. 25kDa and 50kDa; Banding pattern consistent with reference standard	Conform	Conform	Conform
SDS-PAGE (Non-reduced)	Main band approx. 125kDa; Banding pattern consistent with reference standard.	Conform	Conform	Conform

Table 14. Stability after 3 cycles of FT from -60°C or -20±5°C to 5°C of Lot N.1

Stability Indicating Tests	**Specifications**	**Initial T0**	**At < -60°C for T24 followed by 5 ± 3°C for 1 month**	**At < -60°C for T24 followed by 25 ± 2°C for 2 weeks**	**At -20 ± 5°C for T24 followed by + 5 ± 3°C for 1 month**	**At -20 ± 5°C for T24 followed by + 25 ± 2°C for 2 weeks**
Physical appearance of liquid	Colorless to slightly yellowish, clear to slightly opalescent liquid.	Clear and Colorless	Clear, colorless	Clear, colorless	Clear, colorless	Clear, colorless
pH	T0 ± 0.1 (≥ 6.2 and ≤ 6.4)	6.3	6.2	6.2	6.2	6.2
A280	T0 ± 20% (≥ 9.1 mg/mL and ≤ 13.7 mg/mL)	11.4 mg/mL	11.4 mg/mL	11.5 mg/mL	11.4 mg/mL	11.6 mg/mL
SE-HPLC	% Main monomer peak ≥ 95%	98%	98%	95%	98%	95%
CGE (NR)	Comparable to RS	Conform	Conform	Conform	Conform	Conform
	% BEAT: ≥ 90.0%	96.7%	94.8%	96.0%	95.2%	95.8%

CGE (R)	Comparable to RS	Conform	Conform	Conform	Conform	Conform
	Report %LC	17.1%	19.9%	20.6%	19.4%	20.5%
	Report %HC	36.2%	35.0%	36.0%	34.5%	36.2%
	Report %ScFv	46.7%	44.4%	43.2%	45.2%	43.2%
CEX-HPLC	Comparable to RS	Conform	Conform	Conform	Conform	Conform
	Report % Acidic peaks	22.1%	23.5%	24.3%	23.5%	24.2%
	Report % Main peak	25.4%	25.2%	23.5%	25.3%	23.7%
	Report % Basic peaks	52.5%	51.3%	52.1%	51.1%	52.1%
HIC-HPLC	Comparable to RS	Conform	Conform	Conform	Conform	Conform
	Report % BEAT	97.9%	99.7%	99.7%	99.7%	99.7%
cIEF (iCE3)	Comparable to RS	Conform	Conform	Conform	Conform	Conform
	Report % Acidic peaks	22.6%	22.7%	23.3%	22.2%	24.0%
	Report % Main peak	40.8%	42.9%	42.7%	43.7%	42.1%
	Report % Basic peaks	36.6%	34.4%	34.0%	34.1%	33.9%
Binding ELISA	≥ 50% and ≤ 150% of EC50 of Ref. Std.	100%	139%	127%	131%	123%
Functional Assay	≥ 50% and ≤ 150% of EC50 of Ref. Std.	87%	96%	88%	98%	104%
SDS-PAGE	Reduced: Main bands approx. 25kDa and 50kDa; Banding pattern consistent with reference standard	Conform	Conform	Conform	Conform	Conform
	Non-reduced: Main band approx. 125kDa; Banding pattern consistent with reference standard.	Conform	Conform	Conform	Conform	Conform

Table 15. Stability after temperature excursions of Lot N.1

Based on the stability studies made on BEAT®1, Lot N.1 it was concluded that BEAT®1 BDS is stable:

- for 48 months when stored at < -60°C or at -20°C ± 5°C in Charter Medical bag (Freeze-Pak™ STS 50 mL);

- for 1 month when stored at +5°C ± 3°C or +25°C ± 2°C in Charter Medical bag (Freeze-Pak™ STS 50 mL);
- post three freeze-thaw cycles (freezing at < -60°C or at -20°C ± 5°C followed by thawing at +5°C ± 3°C) in Charter Medical bag (Freeze-Pak™ STS 50 mL);
- for 1 month when stored at +5°C ± 3°C and for two weeks at +25°C ± 2°C post thawing from the frozen state (< -60°C or 20°C ± 5°C) for 24 months in Charter Medical bag (Freeze-Pak™ STS 50 mL);

Tests	Specifications	T0	FT -80°C	FT -20°C
pH	T0 ± 0.1 (≥ 6.2 and ≤ 6.4)	6.3	6.4	6.4
Appearance (liquid)	Clear to slightly opalescent, colorless to slightly yellowish	Clear and colorless	Clear and colorless	Clear and colorless
A280	T0 ± 20% (≥ 7.3 mg/mL and ≤ 10.9 mg/mL)	9.1 mg/mL	8.4 mg/mL	8.6 mg/mL
cIEF/iCE3	Comparable to RS. Report as RS	Comparable to RS. 25.8% acidic, 40.7% main, 33.6% basic	Comparable to RS. 27.9% acidic, 41.3% main, 30.8% basic	Comparable to RS. 27.9% acidic, 41.8% main, 30.3% basic
SE-HPLC	% monomer IgG peak ≥ 95%	98%	98%	98%
cGE (NR)	Comparable to RS % of BEAT ≥ 90.0%	96.3%	96.1%	96.7%
CEX-HPLC	Comparable to RS; report % acidic, % main and % basic peak	Comparable to RS; 24.8% acidic, 26.8% main and 48.4% basic	Comparable to RS. 25.5% acidic, 26.9% main, 47.6% basic	Comparable to RS. 25.1% acidic, 27.3% main, 47.6% basic
Binding ELISA	≥ 50 and ≤ 150% of Ref. Std. EC50	126%	101%	121%
Functional Assay	≥ 50 and ≤ 150% of Ref. Std. EC50	89%	88%	NA

Table 16. Stability after 3 cycles of Freeze-Thaw at both -80 ± 20°C and -20 ± 5°C of Lot N.2

Tests	Specifications	Time interval (month)					
		T0 (a)	T1	T2	T3	T6	T9
pH	T0 ± 0.1 (≥ 6.2 and ≤ 6.4)	6.3	6.4	6.4	6.2	6.3	6.2
Appearance (liquid)	Clear to slightly opalescent, colorless to slightly yellowish	Clear and colorless	Clear and colorless	Clear and colorless	Clear and colorless	Clear and colorless	Clear and colorless
A280	T0 ± 20% (≥ 7.3 mg/mL and ≤ 10.9 mg/mL)	9.1 mg/mL	8.6 mg/mL	8.8 mg/mL	9.0 mg/mL	8.6 mg/mL	8.7 mg/mL
cIEF/iCE3	Comparable to RS. Report as RS	Comparable to RS. 25.8% acidic, 40.7% main, 33.6% basic	Comparable to RS. 27.7% acidic, 39.5% main, 32.8% basic	Comparable to RS. 27.3% acidic, 39.1% main, 33.6% basic	Comparable to RS. 25.9% acidic, 42.9% main, 31.2% basic	Comparable to RS. 25.4% acidic, 40.8% main, 33.8% basic	Comparable to RS. 25.6% acidic, 42.7% main, 31.7% basic
SE-HPLC	% monomer IgG peak ≥ 95%	98%	98%	97%	97%	97%	98%
cGE (NR)	Comparable to RS % of BEAT ≥ 90.0%	Comparable to RS BEAT: 96.3%	Comparable to RS BEAT: 96.3%	Comparable to RS BEAT: 96.8%	Comparable to RS BEAT: 93.0%	Comparable to RS BEAT: 96.8%	Comparable to RS BEAT: 96.7%
cGE (R)	Comparable to RS. Report %LC, %HC and %ScFv	NA	NA	NA	NA	Comparable to RS; 20.6% LC, 33.2% HC, 46.2% ScFv	Comparable to RS; 19.6% LC, 35.5% HC, 44.4% ScFv
CEX-HPLC	Comparable to RS; report % acidic, % main and % basic peak	Comparable to RS; 24.8% acidic, 26.8% main and 48.4% basic	Comparable to RS; 25.1% acidic, 27.3% main and 47.5% basic	Comparable to RS; 25.1% acidic, 25.9% main and 49.0% basic	Comparable to RS; 26.3% acidic, 25.7% main, 48.1% basic	Comparable to RS; 25.7% acidic, 27.3% main, 46.9% basic	Comparable to RS; 26.8% acidic, 27.4% main, 45.8% basic
Binding ELISA	≥ 50 and ≤ 150% of Ref. Std. EC50	126%	83%	78%	83%	122%	114%

Functional Assay	≥ 50 and ≤ 150% of Ref. Std. EC50	89%	72%	NA	111%	85%	96%
Bioburden	TC/YM ≤ 1 CFU/mL	TC: 0 CFU/mL YM: 0 CFU/mL	NA	NA	NA	NA	NA
Tests	**Specifications**	**Time interval (month)**					
		T12	**T18**	**T24**	**T36**		
pH	T0 ± 0.1 (≥ 6.2 and ≤ 6.4)	6.2	6.2	6.3	6.2		
Appearance (liquid)	Clear to slightly opalescent, colorless to slightly yellowish	Clear and colorless	Slightly opalescent, colorless	Clear and colorless	Clear and colorless		
A280	T0 ± 20% (≥ 7.3 mg/mL and ≤ 10.9 mg/mL)	8.8 mg/mL	8.5 mg/mL	8.6 mg/mL	8.6 mg/mL		
cIEF/iCE3	Comparable to RS. Report as RS	Comparable to RS. 25.8% acidic, 42.3% main, 31.9% basic	Comparable to RS. 26.2% acidic, 42.1% main, 31.7% basic	Comparable to RS. 24.5% acidic, 42.8% main, 32.8% basic	Comparable to RS. 27.2% acidic, 41.1% main, 31.7% basic		
SE-HPLC	% monomer IgG peak ≥ 95%	99%	95%	98%	97%		
cGE (NR)	Comparable to RS % of BEAT ≥ 90.0%	Comparable to RS BEAT: 96.1%	Comparable to RS BEAT: 96.2%	Comparable to RS BEAT: 95.9%	Comparable to RS BEAT: 96.4%		
cGE (R)	Comparable to RS. Report %LC, %HC and %ScFv	Comparable to RS; 21.3% LC, 33.1% HC, 45.6% ScFv	Comparable to RS; 20.1% LC, 35.6% HC, 43.7% ScFv	Comparable to RS; 20.2% LC, 35.3% HC, 43.9% ScFv	Comparable to RS; 20.8% LC, 34.1% HC, 44.8% ScFv		
CEX-HPLC	Comparable to RS; report % acidic, %	Comparable to RS; 25.8%	Comparable to RS; 24.8%	Comparable to RS; 25.0%	Comparable to RS; 25.7% acidic, 25.6% main, 48.7% basic		

	main and % basic peak	acidic, 24.8% main, 49.4% basic	acidic, 25.3% main, 49.9% basic	acidic, 25.5% main, 49.5% basic	
Binding ELISA	≥ 50 and ≤ 150% of Ref. Std. EC50	102%	87%	105%	84%
Functional Assay	≥ 50 and ≤ 150% of Ref. Std. EC50	93%	125%	83%	92%
Bioburden	TC/YM ≤ 1 CFU/mL	NA	NA	TC: 0 CFU/mL YM: 0 CFU/mL	NA

Table 17. Stability after storage for up to 36 months at -80 ± 20°C of Lot N.2

Test	**Specifications**	**Time interval (months)**				
		T0 (a)	**T1**	**T2**	**T3**	**T6**
pH	T0 ± 0.1 (≥ 6.2 and ≤ 6.4)	6.3	6.3	6.3	6.2	6.3
Appearance (liquid)	Clear to slightly opalescent, colorless to slightly yellowish	Clear and colorless	Clear and colorless	Clear and colorless	Clear and colorless	Clear and colorless
A280	T0 ± 20% (≥ 7.3 mg/mL and ≤ 10.9 mg/mL)	9.1 mg/mL	8.6 mg/mL	8.9 mg/mL	9.1 mg/mL	8.7 mg/mL
cIEF/iCE3	Comparable to RS. Report as RS	Comparable to RS. 25.8% acidic, 40.7% main, 33.6% basic	Comparable to RS. 28.2% acidic, 41.7% main, 30.1% basic	Comparable to RS. 27.6% acidic, 39.1% main, 33.6% basic	Comparable to RS. 26.2% acidic, 25.9% main, 47.8% basic	Comparable to RS. 25.6% acidic, 40.7% main, 33.7% basic
SE-HPLC	% monomer IgG peak ≥ 95%	98%	98%	98%	97%	98%
cGE (NR)	Comparable to RS % of BEAT ≥ 90.0%	Comparable to RS BEAT: 96.3%	Comparable to RS BEAT: 96.3%	Comparable to RS BEAT: 96.4%	Comparable to RS BEAT: 93.3%	Comparable to RS BEAT: 97.0%

cGE (R)	Comparable to RS. Report %LC, %NG and %HC	NA	NA	NA	NA	Comparable to RS; 20.7% LC, 33.3% HC, 46.0% ScFv
CEX-HPLC	Comparable to RS; report % acidic, % main and % basic peak	Comparable to RS; 24.8% acidic, 26.8% main and 48.4% basic	Comparable to RS; 25.1% acidic, 27.2% main and 47.6% basic	Comparable to RS; 25.3% acidic, 26.0% main and 48.7% basic	Comparable to RS; 26.2% acidic, 25.9% main, 47.8% basic	Comparable to RS; 25.4% acidic, 26.2% main, 48.3% basic
Binding ELISA	≥ 50 and ≤ 150% of Ref. Std. EC50	126%	83%	94%	101%	109%
Functional Assay	≥ 50 and ≤ 150% of Ref. Std. EC50	89%	108%	NA	108%	107%
Bioburden	TC/YM ≤ 1 CFU/mL	TC: 0 CFU/mL YM: 0 CFU/mL	NA	NA	NA	NA
Test	**Specifications**	**Time interval (months)**				
		T9	**T12**	**T18**	**T24**	**T36**
pH	T0 ± 0.1 (≥ 6.2 and ≤ 6.4)	6.2	6.2	6.2	6.3	6.2
Appearance (liquid)	Clear to slightly opalescent, colorless to slightly yellowish	Clear and colorless	Clear and colorless	Clear and colorless	Clear and colorless	Clear and colorless
A280	T0 ± 20% (≥ 7.3 mg/mL and ≤ 10.9 mg/mL)	8.7 mg/mL	8.8 mg/mL	8.5 mg/mL	8.7 mg/mL	8.7 mg/mL
cIEF/iCE3	Comparable to RS. Report as RS	Comparable to RS. 25.1% acidic, 43.2% main, 31.7% basic	Comparable to RS. 26.3% acidic, 41.9% main, 31.7% basic	Comparable to RS. 26.2% acidic, 42.5% main, 31.3% basic	Comparable to RS. 24.2% acidic, 42.5% main, 33.2% basic	Comparable to RS. 27.4% acidic, 41.0% main, 31.6% basic
SE-HPLC	% monomer IgG peak ≥ 95%	97%	99%	95%	97%	97%

cGE (NR)	Comparable to RS % of BEAT ≥ 90.0%	Comparable to RS BEAT: 95.7%	Comparable to RS BEAT: 96.1%	Comparable to RS BEAT: 96.4%	Comparable to RS BEAT: 95.6%	Comparable to RS BEAT: 96.3%
cGE (R)	Comparable to RS. Report %LC, %NG and %HC	Comparable to RS; 19.6% LC, 35.3% HC, 44.7% ScFv	Comparable to RS; 21.3% LC, 33.2% HC, 45.6% ScFv	Comparable to RS; 20.2% LC, 35.5% HC, 43.9% ScFv	Comparable to RS; 20.3% LC, 35.5% HC, 43.7% ScFv	Comparable to RS; 20.9% LC, 33.7% HC, 45.2% ScFv
CEX-HPLC	Comparable to RS; report % acidic, % main and % basic peak	Comparable to RS; 26.5% acidic, 27.6% main, 45.9% basic	Comparable to RS; 26.0% acidic, 24.8% main, 49.1% basic	Comparable to RS; 25.1% acidic, 25.2% main, 49.7% basic	Comparable to RS; 25.5% acidic, 24.8% main, 49.6% basic	Comparable to RS; 25.9% acidic, 25.8% main, 48.3% basic
Binding ELISA	≥ 50 and ≤ 150% of Ref. Std. EC50	123%	127%	139%	120%	99%
Functional Assay	≥ 50 and ≤ 150% of Ref. Std. EC50	99%	104%	146%	96%	101%
Bioburden	TC/YM ≤ 1 CFU/mL	NA	NA	NA	TC: 0 CFU/mL YM: 0 CFU/mL	NA

Table 18. Stability after storage for up to 36 months at -20 ± 5°C of Lot N.2

Stability Indicating Tests	**Specifications**	**Time interval (Months)**	
		T0	**T1**
pH	T0 ± 0.1 (≥ 6.2 and ≤ 6.4)	6.3	6.3
Appearance (liquid)	Clear to slightly opalescent, colorless to slightly yellowish	Clear and colorless	Clear and colorless
A280	T0 ± 20% (≥ 7.3 mg/mL and ≤ 10.9 mg/mL)	9.1 mg/mL	8.8 mg/mL
cIEF/iCE3	Comparable to RS. Report as RS	Comparable to RS. 25.8% acidic, 40.7% main, 33.6% basic	Comparable to RS. 29.6% acidic, 41.2% main, 29.1% basic
SE-HPLC	% monomer IgG peak ≥ 95%	98%	98%
cGE (NR)	Comparable to RS % of BEAT ≥ 90.0%	Comparable to RS BEAT: 96.3%	Comparable to RS BEAT: 96.0%

CEX-HPLC	Comparable to RS; report % acidic, % main and % basic peak	Comparable to RS; 24.8% acidic, 26.8% main and 48.4% basic	Comparable to RS; report 26.9% acidic, 26.3% main and 46.8% basic
Binding ELISA	≥ 50 and ≤ 150% of Ref. Std. EC50	126%	103%
Functional Assay	≥ 50 and ≤ 150% of Ref. Std. EC50	89%	NA

Table 19. Stability at + 5±3°C up to 1 month of Lot N.2

Stability Indicating Tests	**Specifications**	**Time interval (Months)**	
		T0	**T1**
pH	T0 ± 0.1 (≥ 6.2 and ≤ 6.4)	6.3	6.3
Appearance (liquid)	Clear to slightly opalescent, colorless to slightly yellowish	Clear and colorless	Clear and colorless
A280	T0 ± 20% (≥ 7.3 mg/mL and ≤ 10.9 mg/mL)	9.1 mg/mL	9.3 mg/mL
cIEF/iCE3	Comparable to RS. Report as RS	Comparable to RS. 25.8% acidic, 40.7% main, 33.6% basic	Comparable to RS. 32.9% acidic, 39.2% main, 27.9% basic
SE-HPLC	% monomer IgG peak ≥ 95%	98%	98%
cGE (NR)	Comparable to RS % of BEAT ≥ 90.0%	Comparable to RS BEAT: 96.3%	Comparable to RS BEAT: 96.1%
CEX-HPLC	Comparable to RS; report % acidic, % main and % basic peak	Comparable to RS; 24.8% acidic, 26.8% main and 48.4% basic	Comparable to RS; report 29.9% acidic, 23.7% main and 46.4% basic
Binding ELISA	≥ 50 and ≤ 150% of Ref. Std. EC50	126%	97%
Functional Assay	≥ 50 and ≤ 150% of Ref. Std. EC50	89%	NA

Table 20. Stability at +25±2°C up to 1 month of Lot N.2

Stability Indicating Tests	Specifications	Time interval		
		T0 (a)	2W -80°C + 1M 5°C	24M -80°C + 1M 5°C
pH	T0 ± 0.1 (≥ 6.2 and ≤ 6.4)	6.3	6.3	6.3
Appearance (liquid)	Clear to slightly opalescent, colorless to slightly yellowish	Clear and colorless	Clear and colorless	Clear and colorless
A280	T0 ± 20% (≥ 7.3 mg/mL and ≤ 10.9 mg/mL)	9.1 mg/mL	9.0 mg/mL	9.0 mg/mL
cIEF/iCE3	Comparable to RS. Report as RS	Comparable to RS. 25.8% acidic, 40.7% main, 33.6% basic	Comparable to RS. 28.8% acidic, 38.0% main, 33.2% basic	Comparable to RS. 27.3% acidic, 41.9% main, 30.8% basic
SE-HPLC	% monomer IgG peak ≥ 95%	98%	98%	98%
cGE (NR)	Comparable to RS % of BEAT ≥ 90.0%	Comparable to RS BEAT: 96.3%	Comparable to RS BEAT: 96.3%	Comparable to RS BEAT: 92.7%
cGE (R)	Comparable to RS. Report %LC, %NG and %HC	NA	NA	Comparable to RS; 20.2% LC, 35.9% HC, 43.4% ScFv
CEX-HPLC	Comparable to RS; report % acidic, % main and % basic peak	Comparable to RS; 24.8% acidic, 26.8% main and 48.4% basic	Comparable to RS; report 26.6% acidic, 25.1% main and 48.2% basic	Comparable to RS; report 25.7% acidic, 25.8% main and 48.4% basic
Binding ELISA	≥ 50 and ≤ 150% of Ref. Std. EC50	126%	120%	83%
Functional Assay	≥ 50 and ≤ 150% of Ref. Std. EC50	89%	NA	88%

Table 21. Stability after freezing at -80°C for 2 weeks or 24M followed by storage for 1 month at + 5 ± 3°C of Lot N.2

Stability Indicating Tests	Specifications	Time interval		
		T0 (a)	2W -20°C + 1M 5°C	24M -20°C + 1M 5°C
pH	T0 ± 0.1 (≥ 6.2 and ≤ 6.4)	6.3	6.3	6.3
Appearance (liquid)	Clear to slightly opalescent, colorless to slightly yellowish	Clear and colorless	Clear and colorless	Clear and colorless

A280	T0 ± 20% (≥ 7.3 mg/mL and ≤ 10.9 mg/mL)	9.1 mg/mL	8.9 mg/mL	7.7 mg/mL
cIEF/iCE3	Comparable to RS. Report as RS	Comparable to RS. 25.8% acidic, 40.7% main, 33.6% basic	Comparable to RS. 29.0% acidic, 38.1% main, 32.9% basic	Comparable to RS. 27.1% acidic, 42.0% main, 30.9% basic
SE-HPLC	% monomer IgG peak ≥ 95%	98%	98%	97%
cGE (NR)	Comparable to RS % of BEAT ≥ 90.0%	Comparable to RS BEAT: 96.3%	Comparable to RS BEAT: 96.2%	Comparable to RS BEAT: 91.9%
cGE (R)	Comparable to RS. Report %LC, %NG and %HC	NA	NA	Comparable to RS; 20.2% LC, 35.5% HC, 43.7% ScFv
CEX-HPLC	Comparable to RS; report % acidic, % main and % basic peak	Comparable to RS; 24.8% acidic, 26.8% main and 48.4% basic	Comparable to RS; report 26.9% acidic, 24.9% main and 48.2% basic	Comparable to RS; report 25.9% acidic, 25.1% main and 49.0% basic
Binding ELISA	≥ 50 and ≤ 150% of Ref. Std. EC50	126%	117%	73%
Functional Assay	≥ 50 and ≤ 150% of Ref. Std. EC50	89%	NA	68%

Table 22. Stability after storage at-20°C for 2 weeks or 24 months followed storage for 1 month at + 5 ± 3°C of Lot N.2

Based on the stability studies made on BEAT®1, Lot N.2 it was concluded that BEAT®1 BDS is stable:

- after 3 cycles of freeze-thaw at both -80 ± 20°C and -20 ± 5°C in Charter Medical bag (Freeze-Pak™ STS 50 mL);
- for 36 months when stored at -80 ± 20°C in Charter Medical bag (Freeze-Pak™ STS 50 mL);
- for 36 months when stored at -20 ± 5°C in Charter Medical bag (Freeze-Pak™ STS 50 mL);
- for 1 month when stored at + 5 ± 3°C in Charter Medical bag (Freeze-Pak™ STS 50 mL);
- for 1 month when stored at + 25 ± 2°C in Charter Medical bag (Freeze-Pak™ STS 50 mL);
- when stored for 2 weeks or 24 months at -80 ± 20°C followed by thawing at + 5 ± 3°C for 1 month in Charter Medical bag (Freeze-Pak™ STS 50 mL);
- when stored for 2 weeks or 24 months at -20 ± 5°C followed by thawing at + 5 ± 3°C for 1 month in Charter Medical bag (Freeze-Pak™ STS 50 mL);

Stability Indicating Tests	Specifications	Time interval (month)						
		T0 (a)	T5	T11	T17	T23	T29	T35
Physical Appearance of liquid	Colorless to slightly yellowish, clear to slightly opalescent liquid	Colorless and clear	Clear and colorless	Clear and colorless	Slightly opalescent, slightly yellowish	Clear and colorless	Clear and colorless	Clear and colorless
pH	T0 ± 0.1 (≥ 6.2 and ≤ 6.4)	6.3	6.2	6.2	6.2	6.2	6.2	6.2
A_{280}	T0 ± 20% (≥ 7.0 mg/mL and ≤ 10.4 mg/mL)	8.7 mg/mL	8.7 mg/mL	8.1 mg/mL	8.6 mg/mL	8.6 mg/mL	8.7 mg/mL	8.6 mg/mL
SE-HPLC	% Main monomer peak ≥ 95%	98%	98%	97%	95%	95%	98%	97%
CGE (NR)	Comparable to RS % of BEAT ≥ 90.0%	Comparable to RS % BEAT: 96.7%	Comparable to RS, % BEAT: 95.9%	Comparable to RS, % BEAT: 95.9%	Comparable to RS, % BEAT: 95.6%	Comparable to RS, % BEAT: 95.7%	Comparable to RS, % BEAT: 96.4%	Comparable to RS, % BEAT: 95.9%
CGE (R)	Comparable to RS	NA	Comparable to RS. 19.7% LC, 35.5% NG, 44.3% HC	Comparable to RS. 21.4% LC, 0.2% NG,31.9 % HC, 46.2% ScFv	Comparable to RS. 20.0% LC, 0.2% NG,35.3% HC, 44.2% ScFv	Comparable to RS. 20.7% LC, 0.2% NG,34.1% HC, 44.8% ScFv	Comparable to RS. 20.2% LC, 33.3% HC, 46.2% ScFv	Comparable to RS. 21.3% LC, 32.2% HC, 46.2% ScFv
CEX-HPLC	Comparable to RS; Report % acidic, % main and % basic peak	Comparable to RS. 24.9% Acidic, 29.8% Main,	Comparable to RS. 25.8% Acidic, 32.1% Main,	Comparable to RS. 26.7% Acidic, 26.1% Main,	Comparable to RS. 25.7% Acidic, 27.5% Main, 46.8% Basic	Comparable to RS. 26.1% Acidic, 27.2% Main,	Comparable to RS. 25.3% Acidic, 29.2% Main,	Comparable to RS. 25.4% Acidic, 28.6% Main,

		45.3% Basic	42.2% Basic	47.2% Basic		46.6% Basic	45.4% Basic	46.0% Basic
cIEF (iCE3)	Comparable to RS, Report as RS	Comparable to RS. 28.6% acidic, 43.3% main, 28.1% basic	Comparable to RS. 26.9% acidic, 44.1% main, 29.0% basic	Comparable to RS. 27.1% acidic, 43.0% main, 30.0% basic	Comparable to RS. 25.8% acidic, 44.6% main, 29.7% basic	Comparable to RS. 26.9% acidic, 43.7% main, 29.5% basic	Comparable to RS. 27.7% acidic, 42.7% main, 29.6% basic	Comparable to RS. 26.4% acidic, 43.2% main, 30.4% basic
Binding ELISA	≥ 50% and ≤ 150% of EC_{50} of Ref. Std.	NA	98%	119%	72%	84%	86%	99%
Functional Assay	≥ 50% and ≤ 150% of EC_{50} of Ref. Std.	79%	96%	121%	86%	117%	117%	86%
Bioburden	TC/YM ≤ 1 CFU/mL	TC: 0 CFU/mL YM: 0 CFU/mL	NA	NA	NA	TC: 0 CFU/mL YM: 0 CFU/mL	NA	NA

Table 23. Stability at -80 ± 20°C up to 35 months for Lot N.3

Tests	**Specifica-tions**	**Time interval (months)**						
		T0 (a)	**T5**	**T11**	**T17**	**T23**	**T29**	**T35**
Physical Appearance of liquid	Colorless to slightly yellowish, clear to slightly opalescent liquid	Colorless, clear and practically free of visual particulates	Clear and colorless	Clear and colorless	Slightly opalescent, slightly yellowish	Clear and colorless	Clear and colorless	Clear and colorless
pH	T0 ± 0.1 (≥ 6.2 and ≤ 6.4)	6.3	6.2	6.2	6.2	6.1 (d)	6.2	6.2
A_{280}	T0 ± 20% (≥ 7.0 mg/mL and ≤ 10.4 mg/mL)	8.7 mg/mL	8.7 mg/mL	8.3 mg/mL	8.5 mg/mL	8.6 mg/mL	8.7 mg/mL	8.7 mg/mL

SE-HPLC	% Main monomer peak ≥ 95%	98%	97%	97%	95%	95%	98%	98%
CGE (NR)	Comparable to RS % of BEAT ≥90.0%	Comparable to RS % BEAT: 96.7%	Comparable to RS, % BEAT: 95.5%	Comparable to RS, % BEAT: 95.9%	Comparable to RS, % BEAT: 95.5%	Comparable to RS, % BEAT: 96.0%	Comparable to RS, % BEAT: 96.3%	Comparable to RS, % BEAT: 96.2%
CGE (R)	Comparable to RS	NA	Comparable to RS. 19.6% LC, 35.2% NG HC, 44.6% HC	Comparable to RS. 20.8% LC,0.3% NG,32.3 % HC, 46.3% ScFv	Comparable to RS. 20.1% LC, 0.2% NG, 35.2% HC, 44.1% ScFv	Comparable to RS. 20.8% LC, 0.1% NG, 34.0% HC, 45.1% ScFv	Comparable to RS. 20.2% LC, 33.3% HC, 46.2% ScFv	Comparable to RS. 20.8% LC, 32.0% HC, 47.0% ScFv
CEX-HPLC	Comparable to RS; report % acidic, % main and % basic peak	Comparable to RS. 24.9% acidic, 29.8% Main, 45.3% Basic	Comparable to RS. 25.9% Acidic, 32.0% Main, 42.1% Basic	Comparable to RS. 26.8% Acidic, 25.4% Main, 47.7% Basic	Comparable to RS. 25.9% Acidic, 27.3% Main, 46.8% Basic	Comparable to RS. 25.3% Acidic, 28.5% Main, 46.3% Basic	Comparable to RS. 25.6% Acidic, 28.8% Main, 45.6% Basic	Comparable to RS. 25.5% Acidic, 28.4% Main, 46.1% Basic
cIEF (iCE3)	Comparable to RS, Report as RS	Comparable to RS28.6% acidic,43.3% main,28.1% basic	Comparable to RS. 26.4% acidic, 44.3% main, 29.3% basic	Comparable to RS. 26.6% acidic, 42.6% main, 30.8% basic	Comparable to RS. 26.2% acidic, 44.2% main, 29.5% basic	Comparable to RS. 27.1% acidic, 43.3% main, 29.6% basic	Comparable to RS. 28.5% acidic, 42.0% main, 29.5% basic	Comparable to RS. 26.9% acidic, 43.0% main, 30.2% basic
Binding ELISA	≥ 50% and ≤ 150% of EC_{50} of Ref. Std.	NA	97%	109%	88%	133%	83%	124%
Functional Assay	≥ 50% and ≤ 150% of EC_{50} of Ref. Std.	79%	107%	69%	81%	115%	105%	107%

Bioburden	TC/YM ≤ 1 CFU/mL	TC: 0 CFU/mL YM: 0 CFU/mL	NA	NA	NA	TC: 0 CFU/mL YM: 0 CFU/mL	NA	NA

Table 24. Stability at -20 ± 5°C up to 35 months for Lot N.3

Based on the stability studies made on BEAT®1, Lot N.1 it was concluded that BEAT®1 BDS is stable:

- for 35 months when stored at -80 ± 20°C;
- for 35 months when stored at -20 ± 5°C.

Example 4: Stability study - DP

The drug product (DP) is formulated in a 5 mM (0.776 mg/mL) L-Histidine buffer containing 5% w/v (50 mg/mL) Sucrose and 0.02% w/v (0.2 mg/mL) Polysorbate 80 (Tween 80). It is presented as a sterile lyophilized powder for injection; lyophilization was carried out as described in Table 2. Post reconstitution with WFI, the solution contains GBR 1302 at a concentration of 0.1 mg/mL at a pH of 6.3. Required no. of BEAT®1 DP vials were stored under various temperature conditions of +5 ± 3 °C (Table 25), +25 ± 2 °C (Table 26) and +40 ± 2 °C (Table 27) in order to assess its stability.

Tests	**Specifications**	**Time interval (months)**					
		Initial (T0)	**T1**	**T2**	**T3**	**T6**	**T9**
Cake appea-rance	Report results (uniformity, adherence to the vial surface, flatness and presence or not of small cracks on the surface)	Uniform, adherent to most of the surface, flat, not cracked or small cracks on the surface	Uniform, adherent to most of the surface, flat, not cracked or small cracks on the surface	Uniform, adherent to most of the surface, flat, not cracked or small cracks on the surface	Uniform, adherent to most of the surface, flat, not cracked or small cracks on the surface	Uniform, not adherence to the vial surface, flat, small crack on the surface	Uniform, flat, not adherent to vial surface, no cracks on the surface
Reconstit ution time	Report Result (sec)	36	29	98	103	30	45
Residual moisture content (Karl Fisher)	≤ 3%	1%	NA	NA	1%	NA	NA

Physical appearance of liquid	Colorless to slightly yellowish, clear to slightly opalescent liquid.	Clear and colorless	Clear and colorless	Clear and colorless	Clear and colorless	Clear and colorless	Clear and colorless
pH	T0 ± 0.1 (≥ 6.2 and ≤ 6.4)	6.3	6.2	6.3	6.3	6.2	6.3
A280	T0 ± 20%(≥ 0.06 mg/mL and ≤ 0.10 mg/mL)	0.08	0.06	0.09	0.08	0.09	0.08
SE-HPLC	% Main monomer peak ≥ 95%	97%	97%	98%	98%	98%	98%
SDS-PAGE (NR)	Non-reduced: Main band approx. 125kDa; Banding pattern consistent with reference standard.	Conform	Conform	Conform	Conform	NA	Conform
SDS-PAGE (R)	Reduced: Main bands approx. 25kDa and 50kDa; Banding pattern consistent with reference standard	Conform	Conform	Conform	Conform	NA	Conform
CGE (NR)	Comparable to RS. Report results.	Comparable to RS, BEAT: 97%	Comparable to RS, BEAT: 97%	Comparable to RS, BEAT: 97%	Comparable to RS, BEAT: 97%	Comparable to RS, BEAT: 97%	Comparable to RS, BEAT: 97%
CGE (R)	Comparable to RS. Report results.	NT	NT	NT	NT	Conform	NA
CEX-HPLC	Comparable to RS, Report % acidic, % Main	Comparable to RS, % Acidic 23.3	Comparable to RS, % Acidic 22.3	Comparable to RS, %Acidic:	Comparable to RS, %Acidic:	Comparable to RS,	NA

	and % Basic peaks	% Main: 26.9 % Basic: 49.9	% Main: 28.2 % Basic: 49.6	21.5 %Main: 27.3 % Basic: 51.1	22.9 %Main: 28.2 % Basic: 48.8	% Acidic: 23.1 % Main: 27.9 % Basic: 49.0	
cIEF (iCE3)	Comparable to RS. Report as RS.	Comparable to RS, Acidic 23.6%Main: 41.4%Basic: 35.0%	Comparable to RS, Acidic 26.3%Main: 38.5%Basic: 35.3%	Comparable to RS, Acidic 24.6%Main: 38.6%Basic: 36.8%	Comparable to RS, Acidic 26.7%Main: 40.1%Basic: 33.2%	Comparable to RS, Acidic: 24.6%, Main: 40.6%Basic :34.4%	Not comparable to RS, Acidic 28.7%Main: 39.3%Basic: 32.0%
Binding ELISA	≥ 50% and ≤ 150% of RS EC50	100	54	86	77	78	102
Functional assay	≥ 50% and ≤ 150% of RS EC50	NT	NT	84	74	83	81
Sub-visible particles	NMT 600 ≥25µm per container	6 particles per container	NA	NA	NA	NA	NA
	NMT 6000 ≥10µm per container	70 particles per container	NA	NA	NA	NA	NA
Tests	**Specifications**	**Time interval (months)**					
		T12	**T18**	**T24**	**T30**	**T36**	
Cake appearance	Report results (uniformity, adherence to the vial surface, flatness and presence or not of small cracks on the surface)	Uniform, flat, not adherent to vial surface, no visible cracks	Uniform, flat, adherent to the vial surface, no visible cracks	Uniform, Not flat/Convex, not adherent to the vial surface, no visible cracks	Uniform, flat, not adherent to vial surface, flat, no visible cracks (d)	Uniform, flat with volcano, not adherent to vial surface, flat, no visible cracks (d)	
Reconstitution time	Report Result (sec)	20	25	28	20	75	
Residual moisture content	≤ 3%	1%	NA	2%	NA	1%	

(Karl Fisher)						
Physical appearance of liquid	Colorless to slightly yellowish, clear to slightly opalescent liquid.	Clear and colorless	Clear and colorless	Clear and colorless	Clear and colorless	Clear and colorless
pH	T0 ± 0.1 (≥ 6.2 and ≤ 6.4)	6.3	6.2	6.3	6.2	6.3
A280	T0 ± 20%(≥ 0.06 mg/mL and ≤ 0.10 mg/mL)	0.08	0.09	0.09	0.09	0.09
SE-HPLC	% Main monomer peak ≥ 95%	98%	96%	98%	97%	98%
SDS-PAGE (NR)	Non-reduced: Main band approx. 125kDa; Banding pattern consistent with reference standard.	NA	NA	NA	NA	NA
SDS-PAGE (R)	Reduced: Main bands approx. 25kDa and 50kDa; Banding pattern consistent with reference standard	NA	NA	NA	NA	NA
CGE (NR)	Comparable to RS. Report results.	Comparable to RS, BEAT: 95%	Comparable to RS, BEAT: 97%	Comparable to RS, BEAT: 96%	Comparable to RS, BEAT: 95%	Comparable to RS BEAT:96%
CGE (R)	Comparable to RS. Report results.	Conform	Conform	Conform	Conform	Conform
CEX-HPLC	Comparable to RS, Report %	Comparable to RS,	Comparable to RS,	Comparable to RS,	Comparable to RS,	Comparable to RS, %Acidic: 22.8

	acidic, % Main and % Basic peaks	% Acidic25.4 %Main: 27.1 %Basic: 47.5	%Acidic: 23.9 %Main: 28.4 %Basic: 47.7	%Acidic: 23.6 %Main: 26.1 %Basic: 50.2	%Acidic: 23.1 %Main: 26.9 %Basic: 50.0	%Main: 27.4 %Basic:49.8
cIEF (iCE3)	Comparable to RS. Report as RS.	Comparable to RS, Acidic 22.9%Main: 40.8, Basic: 36.3%	Comparable to RS, Acidic 26.6% Main: 41.6% Basic: 31.8%	Comparable to RS, Acidic 22.7% Main: 43.4% Basic: 33.9%	Comparable to RS, Acidic 24.2% Main: 41.3% Basic: 34.6%	Comparable to RS, Acidic 25.9% Main: 40.8% Basic: 33.3%
Binding ELISA	≥ 50% and ≤ 150% of RS EC50	82	75	136	76	101
Functional assay	≥ 50% and ≤ 150% of RS EC50	105	99	62	149	112
Sub-visible particles	NMT 600 ≥25µm per container	11 particles per container	NA	17 particles per container	NA	0 particles per container
	NMT 6000 ≥10µm per container	28 particles per container	NA	380 particles per container	NA	6 particles per container

Table 25. Stability at +5 ± 3°C up to 36 months

Tests	**Specifications**	**Time interval (months)**				
		Initial (T0)	**T1**	**T2**	**T3**	**T6**
Cake appearance	Report results (uniformity, adherence to the vial surface, flatness and presence or not of small cracks on the surface)	Uniform, adherent to most of the surface, flat, not cracked or small cracks on the surface	Uniform, adherent to most of the surface, flat, not cracked or small cracks on the surface	Uniform, adherent to most of the surface, flat, not cracked or small cracks on the surface	Uniform, adherent to most of the surface, flat, not cracked or small cracks on the surface	Uniform, not adherence to the vial surface, flat, no crack on the surface

Reconstitution time	Report Result (sec)	36	62	86	66	27
Residual moisture content (Karl Fisher)	≤ 3%	1%	NA	NA	2%	NA
Physical appearance of liquid	Colorless to slightly yellowish, clear to slightly opalescent liquid	Clear and color-less	Clear and color-less	Clear and color-less	Clear and color-less	Clear and color-less
pH	T0 ± 0.1 (≥ 6.2 and ≤ 6.4)	6.3	6.2	6.3	6.3	6.2
A280	T0 ± 20% (≥ 0.06 mg/mL and ≤ 0.10 mg/mL)	0.08	0.06	0.09	0.08	0.09
SE-HPLC	% Main monomer peak ≥ 95%	97%	97%	98%	98%	98%
SDS-PAGE (NR)	Non-reduced: Main band approx. 125kDa; Banding pattern consistent with reference standard.	Conform	Conform	Conform	Conform	NA
SDS-PAGE (R)	Reduced: Main bands approx. 25kDa and 50kDa; Banding pattern consistent with reference standard	Conform	Conform	Conform	Conform	NA
CGE (NR)	Comparable to RS. Report results	Comparable to RS, BEAT: 97%	Comparable to RS, BEAT: 97%	Comparable to RS, BEAT: 97%	Comparable to RS, BEAT: 97%	Comparable to RS, BEAT: 97%
CGE (R)	Comparable to RS. Report results	NT	NT	NT	NT	Conform

CEX-HPLC	Comparable to RS, Report % acidic, % Main and % Basic peaks	Comparable to RS, %Acidic: 23.3 %Main: 26.9 %Basic: 49.9	Comparable to RS, %Acidic: 22.4 %Main: 28.3 %Basic: 49.2	Comparable to RS, %Acidic: 21.2 %Main: 27.9 %Basic: 51.0	Comparable to RS, %Acidic: 22.9 %Main: 27.9 %Basic: 49.1	Comparable to RS, %Acidic: 23.4 %Main: 28.0 %Basic: 48.6
cIEF (iCE3)	Comparable to RS. Report as RS	Comparable to RS, Acidic: 23.6%, Main: 41.4%, Basic: 35.0%	Comparable to RS, Acidic: 25.5%, Main: 39.3%, Basic: 35.2%	Comparable to RS, Acidic: 24.2%, Main: 39.5%, Basic: 36.3%	Comparable to RS, Acidic: 25.8%, Main: 40.5%, Basic: 33.6%	Comparable to RS, Acidic: 24.5%, Main: 43.0%, Basic:32.4%
Binding ELISA	≥ 50% and ≤ 150% of RS EC50	100	51	102	69	83
Functional assay	≥ 50% and ≤ 150% of RS EC50	NT	NT	NT	79	97
Sub-visible particles	NMT 600 ≥25µm per container	6 particles per conta-iner	NA	NA	NA	NA
	NMT 6000 ≥10µm per container	70 particles per conta-iner	NA	NA	NA	NA
Tests	**Specifications**	**Time interval (months)**				
		T9	**T12**	**T18**	**T24**	
Cake appearance	Report results (uniformity, adherence to the vial surface, flatness and presence or not of small cracks on the surface)	Uniform, flat, not adherent to vial surface, no cracks on the surface	Uniform, flat, not adherent to vial surface, no visible cracks	Uniform, flat, adherent to the vial surface, no visible cracks	Uniform, Not flat/Convex, Not Adherent to the vial surface, no visible cracks	
Reconstitution time	Report Result (sec)	47	20	32	32	
Residual moisture	≤ 3%	NA	1%	NT	3%	

content (Karl Fisher)					
Physical appearance of liquid	Colorless to slightly yellowish, clear to slightly opalescent liquid	Clear and color-less	Clear and color-less	Clear and color-less	Clear and color-less
pH	T0 ± 0.1 (≥ 6.2 and ≤ 6.4)	6.3	6.3	6.2	6.2
A280	T0 ± 20% (≥ 0.06 mg/mL and ≤ 0.10 mg/mL)	0.08	0.08	0.08	0.09
SE-HPLC	% Main monomer peak ≥ 95%	98%	98%	98%	98%
SDS-PAGE (NR)	Non-reduced: Main band approx. 125kDa; Banding pattern consistent with reference standard.	Conform	NA	NA	NA
SDS-PAGE (R)	Reduced: Main bands approx. 25kDa and 50kDa; Banding pattern consistent with reference standard	Conform	NA	NA	NA
CGE (NR)	Comparable to RS. Report results	Comparable to RS, BEAT: 96%	Comparable to RS, BEAT: 96%	Comparable to RS, BEAT: 97%	Comparable to RS, BEAT: 96%
CGE (R)	Comparable to RS. Report results	NA	Conform	Conform	Conform
CEX-HPLC	Comparable to RS, Report % acidic, % Main and % Basic peaks	NA	Comparable to RS, %Acidic: 25.4 %Main:	Comparable to RS, %Acidic: 23.3 %Main: 28.0	Comparable to RS, %Acidic: 23.6 %Main: 25.9 %Basic:50.5

			27.4 %Basic: 47.2	%Basic: 48.7	
cIEF (iCE3)	Comparable to RS. Report as RS	Comparable to RS, Acidic: 28.3%, Main: 38.9%, Basic:32.8%	Comparable to RS, Acidic: 24.6%, Main: 42.4%, Basic: 33.0%	Comparable to RS, Acidic: 26.8%, Main: 40.8%, Basic: 32.4%	Comparable to RS, Acidic: 22.8% Main: 42.9% Basic: 34.3%
Binding ELISA	≥ 50% and ≤ 150% of RS EC50	105	85	86	101
Functional assay	≥ 50% and ≤ 150% of RS EC50	109	118	77	88
Sub-visible particles	NMT 600 ≥25µm per container	NA	0 particles per conta-ner	NA	0 particles per conta-iner
	NMT 6000 ≥10µm per container	NA	11 particles per conta-iner	NA	281 particles per conta-iner

Table 26. Stability at +25 ± 2°C up to 24 months

Stability Indicating Tests	**Specifications**	**Time Interval (months)**			
		Initial (T0)	**T1**	**T2**	**T3**
Cake appearance	Report results (uniformity, adherence to the vial surface, flatness and presence or not of small cracks on the surface)	Uniform, adherent to most of the surface, flat, not cracked or small cracks on the surface	Uniform, adherent to most of the surface, flat, not cracked or small cracks on the surface	Uniform, adherent to most of the surface, flat, not cracked or small cracks on the surface	Uniform, adherent to most of the surface, flat, not cracked or small cracks on the surface
Reconstitution time	Report Result (sec)	36	60	25	59
Residual moisture content (Karl Fisher)	≤ 3%	1%	NA	NA	2%
Physical appearance of liquid	Colourless to slightly yellowish, clear to slightly opalescent liquid.	Clear and colorless	Clear and colorless	Clear and colorless	Clear and colorless
pH	T0 ±0.1 (≥ 6.2 and ≤ 6.4)	6.3	6.2	6.3	6.3

A280	T0 ± 20% (≥ 0.06 mg/mL and ≤ 0.10 mg/mL)	0.08	0.06	0.09	0.08
SE-HPLC	% Main monomer peak ≥ 95%	97%	98%	98%	98%
SDS-PAGE (NR)	Non-reduced: Main band approx. 125kDa; Banding pattern consistent with reference standard	Conform	Conform	Conform	Conform
SDS-PAGE (R)	Reduced: Main bands approx. 25kDa and 50kDa; Banding pattern consistent with reference standard	Conform	Conform	Conform	Conform
CGE (NR)	Comparable to RS. Report results	Comparable to RS, BEAT: 97%	Comparable to RS, BEAT: 97%	Comparable to RS, BEAT: 97%	Comparable to RS, BEAT: 97%
CEX-HPLC	Comparable to RS, Report % acidic, % Main and % Basic peaks	Comparable to RS, %Acidic: 23.3 %Main: 26.9 %Basic: 49.9	Comparable to RS, %Acidic: 22.5 %Main: 27.9 %Basic: 49.5	Comparable to RS, %Acidic: 21.8 %Main: 27.2% Basic: 51.0	Comparable to RS, %Acidic: 23.0 %Main: 27.7 %Basic: 49.3
cIEF (iCE3)	Comparable to RS. Report as RS	Comparable to RS, Acidic: 23.6%, Main: 41.4%, Basic: 35.0%	Comparable to RS, Acidic: 26.2%, Main: 38.3%, Basic: 35.4%	Comparable to RS, Acidic: 25.5%, Main: 38.6%, Basic: 35.9%	Comparable to RS, Acidic: 26.8%, Main: 38.8%, Basic: 34.4%
Binding ELISA	≥ 50% and ≤ 150% of RS EC50	100	66	83	118
Functional assay	≥ 50% and ≤ 150% of RS EC50	NT	NT	NT	86
Sub-visible particles	NMT 600 ≥25µm per container	6 particles per container	NA	NA	0 particles per container
	NMT 6000 ≥10µm per container	70 particles per container	NA	NA	4 particles per container

Table 27. Stability at +40 ± 2°C up to 3 months

BEAT®1 DP is found to be within specification for all the analytical assays for 36 months when stored at +5 ± 3 °C and 24 months when stored at +25 ± 2 °C.

The DP conformed to criteria of purity (based on SE-HPLC, SDS-PAGE and cGE non-reduced and reduced), charge profile (based on iCE3 and CEX-HPLC), protein content (A280), sub-visible particles, moisture content (Karl Fisher) and binding to target (Binding ELISA and Functional Assay). No significant increase in the reconstitution time of lyophilized cake was observed during stability period. The physical appearance and pH values of reconstituted solution confirmed to be within the predefined specifications. The

lyophilized cake appeared uniform with no visible cracks but had loosen its contact with vial surface at the end of 6 months of storage under +5 ± 3 °C and 6 months of storage at +25 ± 2 °C without adversely impacting the product quality.

Nine month (T9M) data point at +5 ± 3 °C did not showed comparable profile as to RS in iCE3 analysis but subsequent all time points were not only having similar profile but also showed comparable % of all charged species.

These data show that BEAT®1 DP is stable:

- for 36 months at +5°C ± 3°C
- for 24 months at +25°C ± 2°C
- for 3 months at +40°C ± 2°C

CLAIMS

1. A stable pharmaceutical formulation comprising a bispecific antibody or an antibody fragment thereof, a buffer and one or more stabilizing or tonicity agents.

2. The pharmaceutical formulation of claim 1, wherein said pharmaceutical formulation is liquid, or lyophilized or reconstituted.

4. The pharmaceutical formulation of any one of the preceding claims, wherein said antibody or antibody fragment thereof is present within said pharmaceutical formulation at a concentration between 0.05 mg/mL and 15 mg/mL.

5. The pharmaceutical formulation of any one of the preceding claims, wherein said pharmaceutical formulation has a pH between 5.5 and 7.0.

6. The pharmaceutical formulation of any one of the preceding claims wherein said buffer is selected from the group comprising acetate, L-histidine, citrate and phosphate.

7. The pharmaceutical formulation of claim 6 wherein said buffer is histidine, present within said pharmaceutical formulation at a concentration comprised between 1 mM and 20 mM.

8. The pharmaceutical formulation of any one of the preceding claims, wherein said stabilizing or tonicity agent is selected from the group comprising sodium acetate, sodium bicarbonate, sodium carbonate, sodium chloride, potassium acetate, potassium bicarbonate, potassium carbonate, potassium chloride, calcium chloride, sucrose, glutamate, mannitol, polyols, Polysorbate 20, Polysorbate 40, Polysorbate 80, Poloxamer, Poloxamer 188, Poloxamer 407, amino acids such as histidine, arginine, glycine, methionine, proline, lysine, glutamic acid, amines, cyclodextrins, β-cyclodextrins, polyvinylpyrrolidone, polyethylene glycol 400, sorbitol, trehalose and EDTA, present within said pharmaceutical formulation at a percentage between about 0.005% and about 20%.

9. The pharmaceutical formulation of claim 8, wherein said stabilizing or tonicity agent is sucrose present within said pharmaceutical formulation at a concentration comprised between about 2% and about 15%; and/or mannitol present within said pharmaceutical formulation at a concentration comprised between about 2% and about 6%; and/or glycine present within said pharmaceutical formulation at a concentration comprised between about 0.4% and about 1.2%; and/or Polysorbate 80 present within said pharmaceutical formulation at a concentration comprised between about 0.01% and about 0.11%; and/or arginine present within said pharmaceutical formulation at a concentration of about 0.4%; and/or

glutamate present within said pharmaceutical formulation at a concentration of about 0.4%; and/or polyvinylpyrrolidone present within said pharmaceutical formulation at a concentration of about 0.1%; and/or β-cyclodextrin present within said pharmaceutical formulation at a concentration of about 0.1%.

10. The pharmaceutical formulation of any one of the preceding claims wherein said pharmaceutical formulation comprises said bispecific antibody or fragment thereof present within said pharmaceutical formulation at a concentration selected from the group comprising about 0.1 mg/mL, about 0.3 mg/mL , about 1 mg/mL and about 10±2 mg/mL, histidine buffer present within said pharmaceutical formulation at a concentration of about 5 mM, sucrose present within said pharmaceutical formulation at a percentage of about 5% and Polysorbate 80 present within said pharmaceutical formulation at a percentage of about 0.02%, and wherein said pharmaceutical formulation has pH of about 6.3.

11. The pharmaceutical formulation of claim 10, wherein said bispecific antibody or fragment thereof is present within said pharmaceutical formulation at a concentration of about 10±2 mg/mL and said pharmaceutical formulation is liquid.

12. The pharmaceutical formulation of claim 10, wherein said bispecific antibody or fragment thereof is present within said pharmaceutical formulation at a concentration of about 0.1 mg/mL and said pharmaceutical formulation is lyophilized or reconstituted.

13. The pharmaceutical formulation of claim 11, wherein said pharmaceutical formulation is stable at about 25°C for at least 1 month; and/or at about 5°C for at least 1 month; and/or at about -20°C for at least 48 months; at about -60°C for at least 48 months; and/or at about -80°C for at least 36 months.

14. The pharmaceutical formulation of claim 12, wherein said pharmaceutical formulation is stable at about 40°C for at least 3 months; and/or at about 25°C for at least 24 months; and/or at about 5°C for at least 36 months.

15. The pharmaceutical formulation of any one of the preceding claims wherein said bispecific antibody or an antibody fragment thereof binds CD3 and HER2.

16. The pharmaceutical formulation of any one of the preceding claims wherein said bispecific antibody or an antibody fragment thereof comprises the amino acid sequences of SEQ ID NOs: 2, 4 and 6.

17. A method of manufacturing the pharmaceutical formulation of any one of the preceding claims.

18. A method to test the pharmaceutical formulation of any one of the preceding claims to any assay for stability determination.

19. An article of manufacture comprising the pharmaceutical formulation of any one of the preceding claims.

FIG. 1A

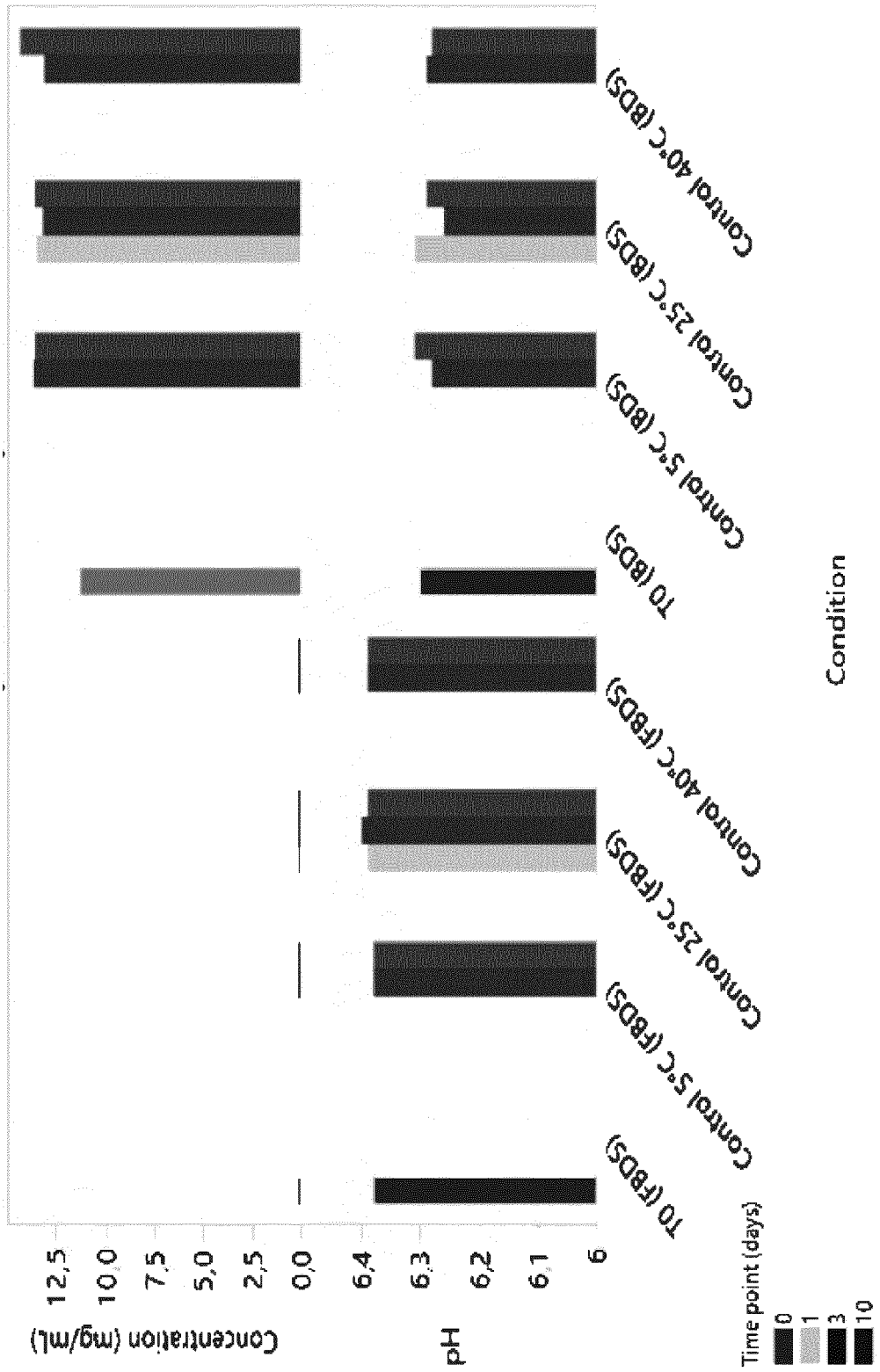

Concentration (mg/mL)
12,5
10,0
7,5
5,0
2,5
0,0
pH
6,4
6,3
6,2
6,1
6
T0 (FBDS)
Control 5°C (FBDS)
Control 25°C (FBDS)
Control 40°C (FBDS)
T0 (BDS)
Control 5°C (BDS)
Control 25°C (BDS)
Control 40°C (BDS)
Condition
Time point (days)
0
1
3
10

FIG. 1B

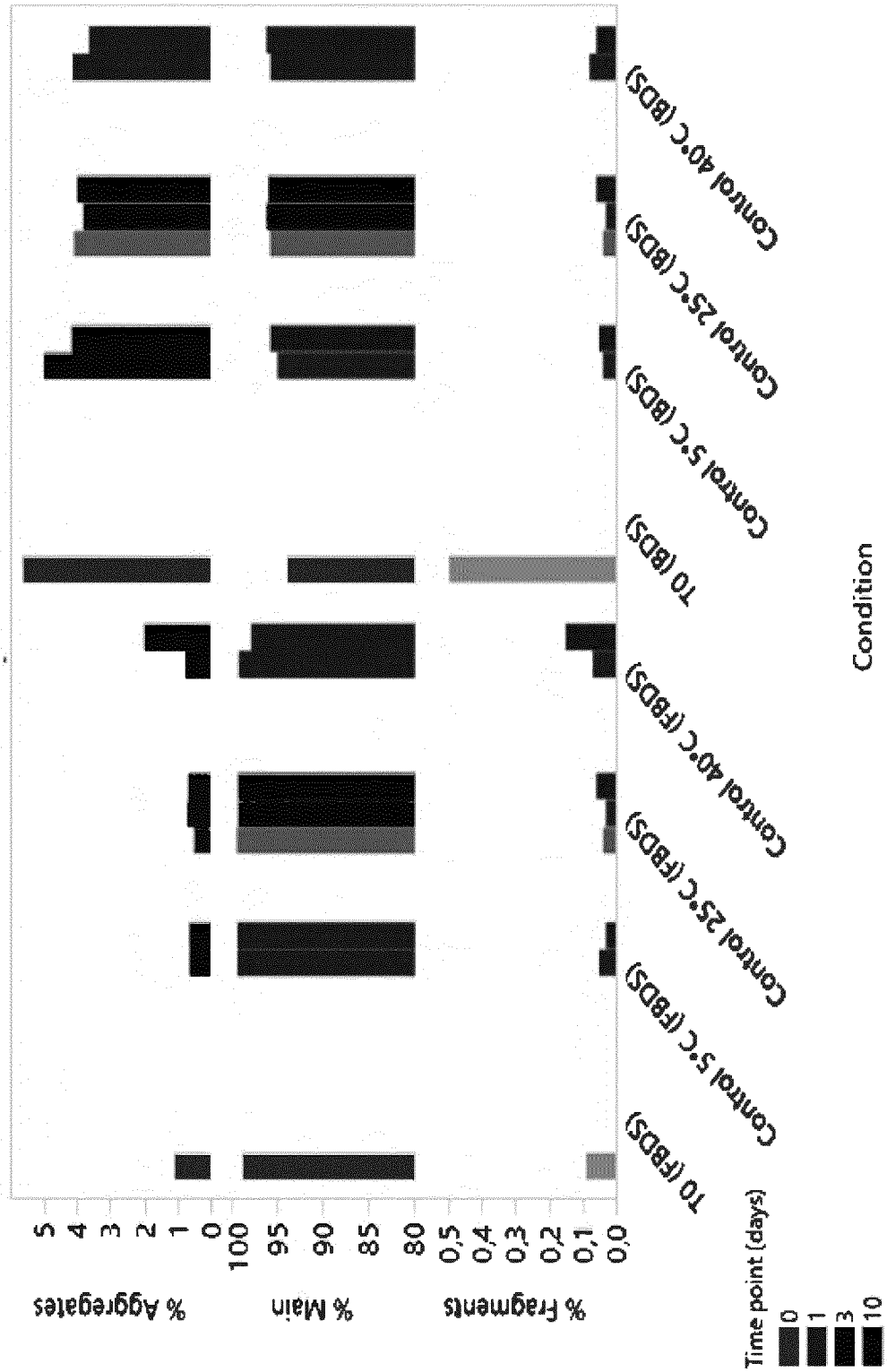

% Aggregates
% Main
% Fragments
Time point (days)
0
1
3
10
Condition
T0 (FBDS)
Control 5°C (FBDS)
Control 25°C (FBDS)
Control 40°C (FBDS)
T0 (BDS)
Control 5°C (BDS)
Control 25°C (BDS)
Control 40°C (BDS)

FIG. 2A

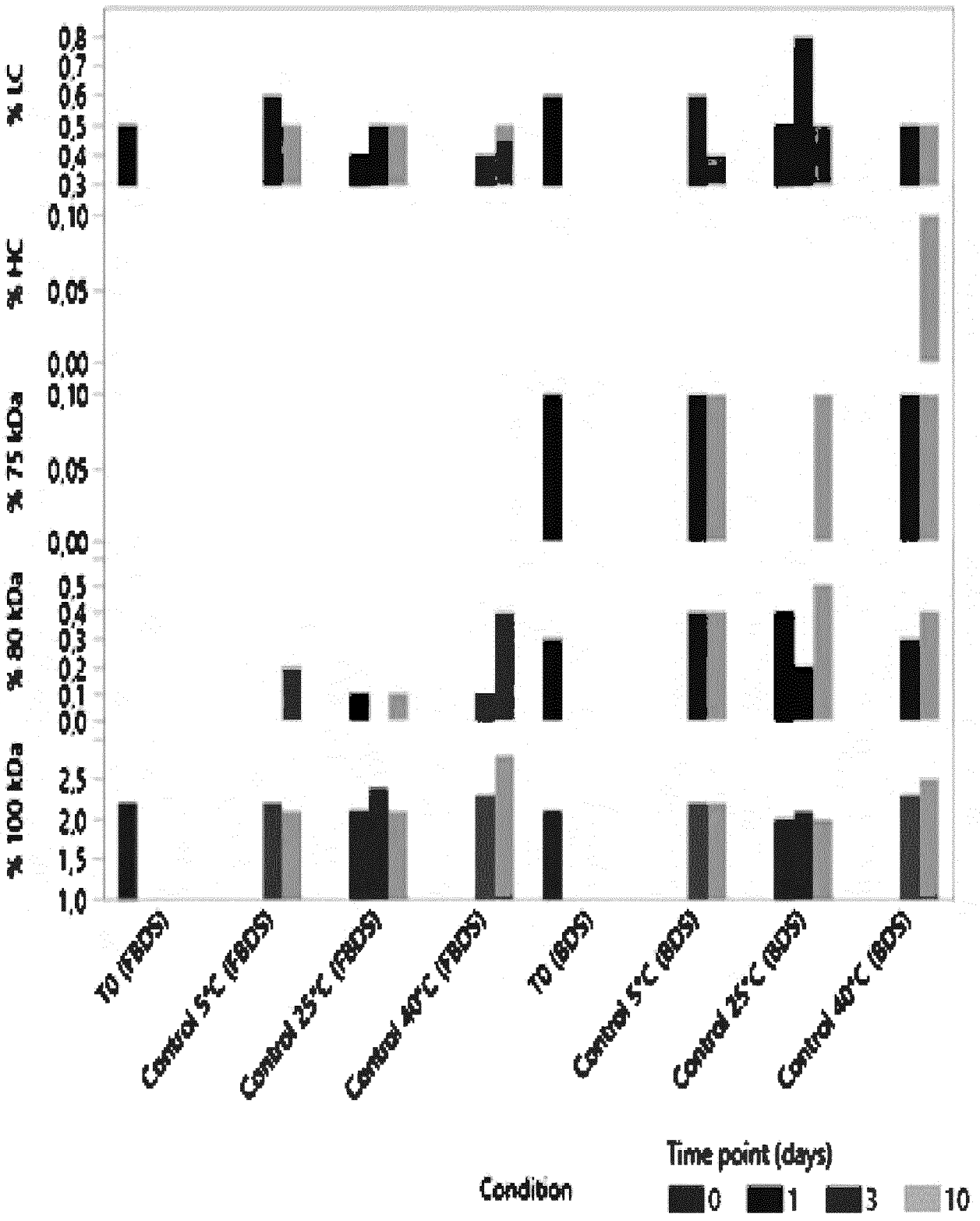

% LC
% HC
% 75 kDa
% 80 kDa
% 100 kDa
T0 (FBDS)
Control 5°C (FBDS)
Control 25°C (FBDS)
Control 40°C (FBDS)
T0 (BDS)
Control 5°C (BDS)
Control 25°C (BDS)
Control 40°C (BDS)
Condition
Time point (days)
0
1
3
10

FIG. 2B

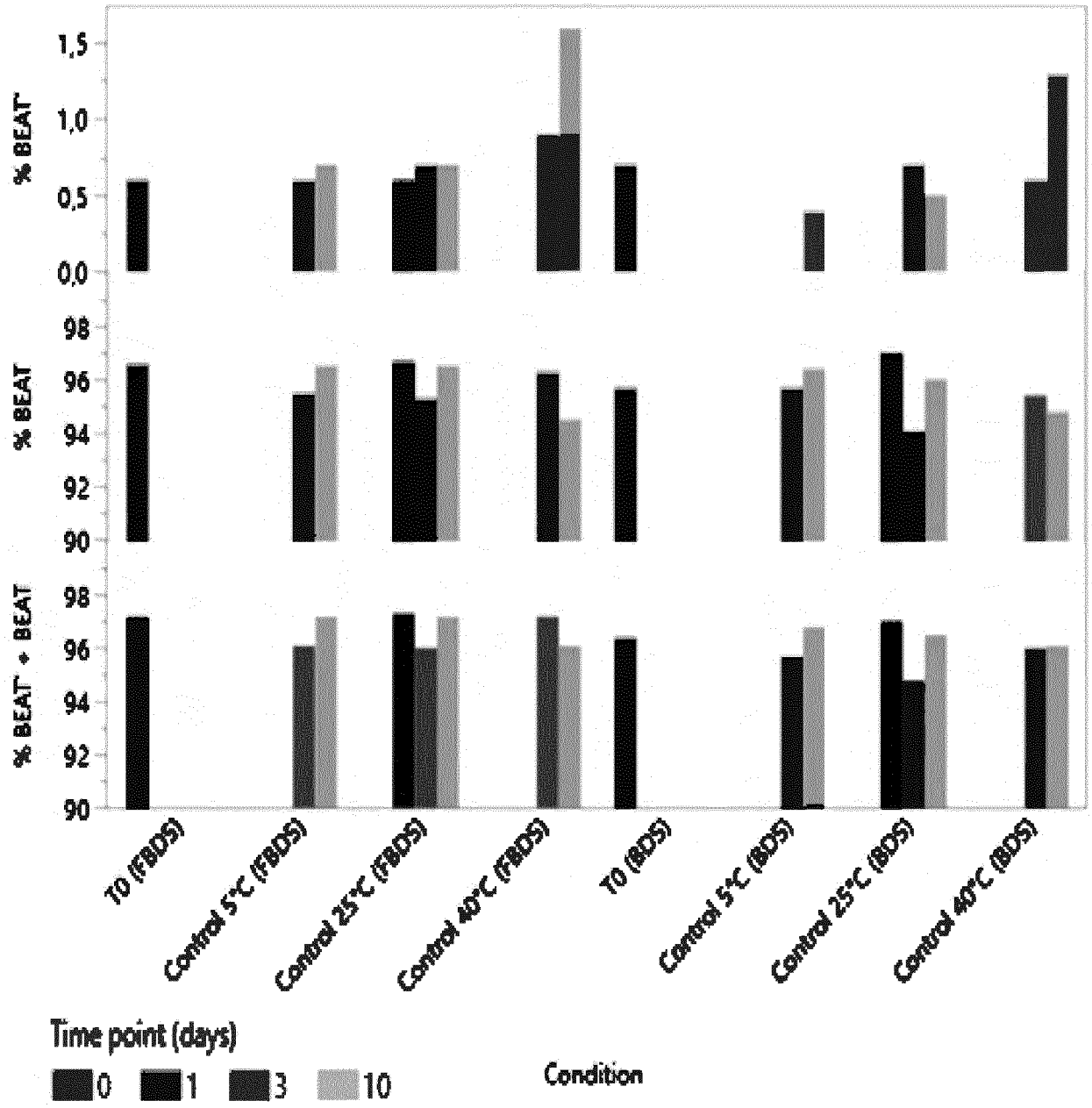

% BEAT
1,5
1,0
0,5
0,0
% BEAT
98
96
94
92
90
% BEAT + BEAT
98
96
94
92
90
T0 (FBDS)
Control 5°C (FBDS)
Control 25°C (FBDS)
Control 40°C (FBDS)
T0 (BDS)
Control 5°C (BDS)
Control 25°C (BDS)
Control 40°C (BDS)
Time point (days)
0
1
3
10
Condition

FIG. 3

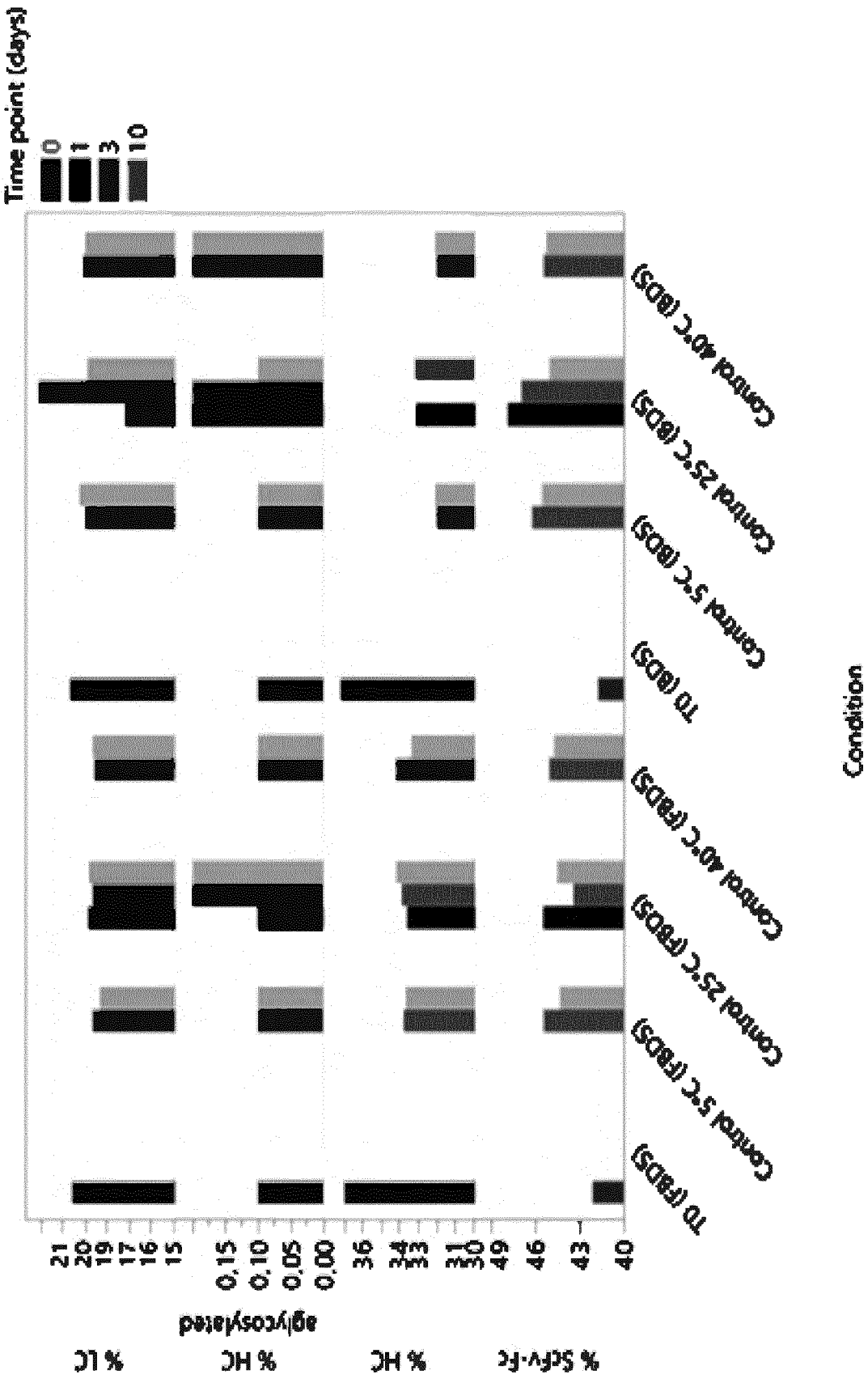

Time point (days)
0
1
3
10
% LC
% HC
% HC
% scFv-Fc
aglycosylated
21
20
19
17
16
15
0,15
0,10
0,05
0,00
36
34
33
31
30
49
46
43
40
TO (FBDS)
Control 5°C (FBDS)
Control 25°C (FBDS)
Control 40°C (FBDS)
TO (BDS)
Control 5°C (BDS)
Control 25°C (BDS)
Control 40°C (BDS)
Condition

FIG. 4

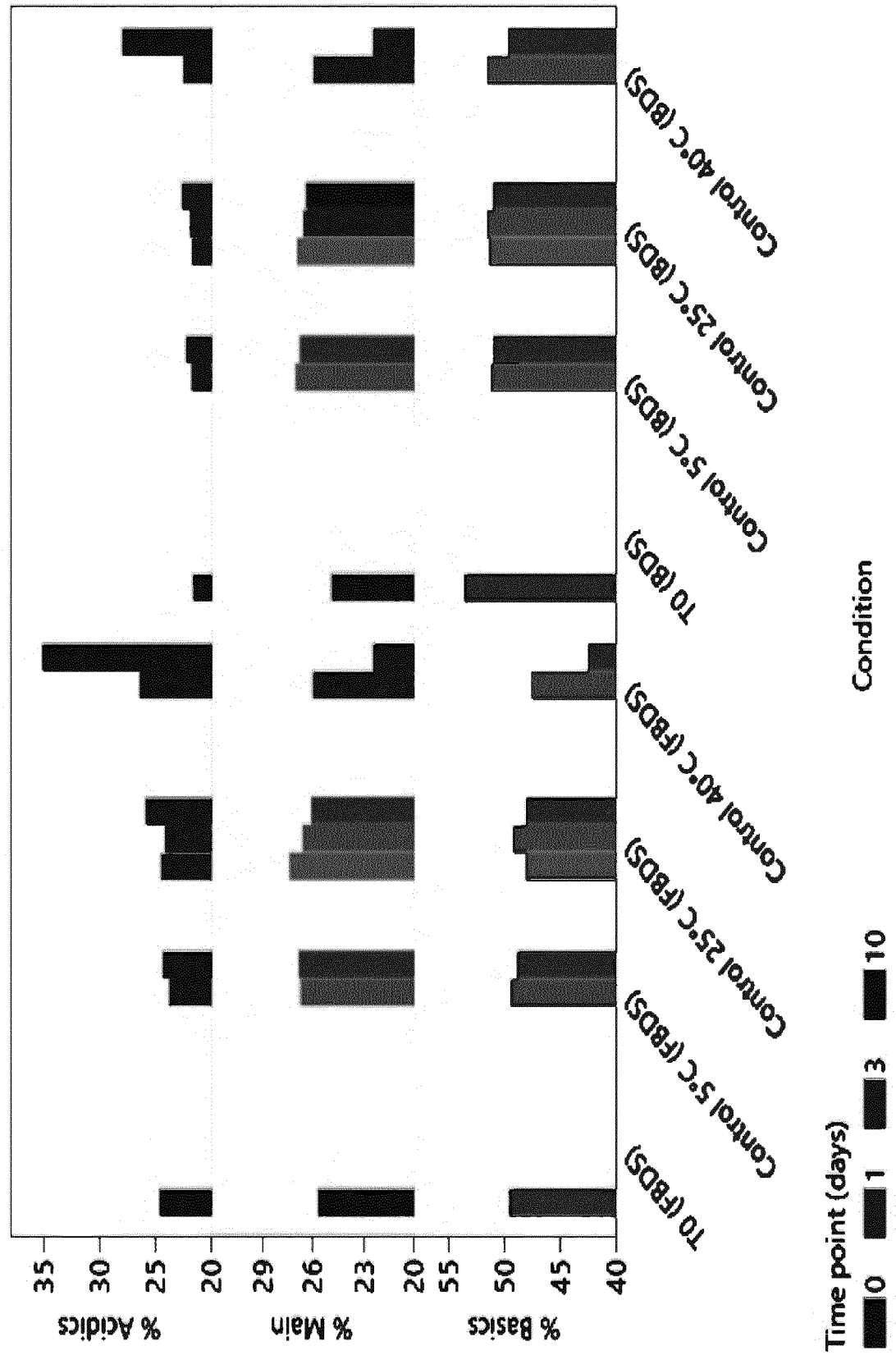

% Acidics
% Main
% Basics
35
30
25
20
29
26
23
20
55
50
45
40
T0 (FBDS)
Control 5°C (FBDS)
Control 25°C (FBDS)
Control 40°C (FBDS)
T0 (BDS)
Control 5°C (BDS)
Control 25°C (BDS)
Control 40°C (BDS)
Condition
Time point (days)
0
1
3
10

FIG. 5

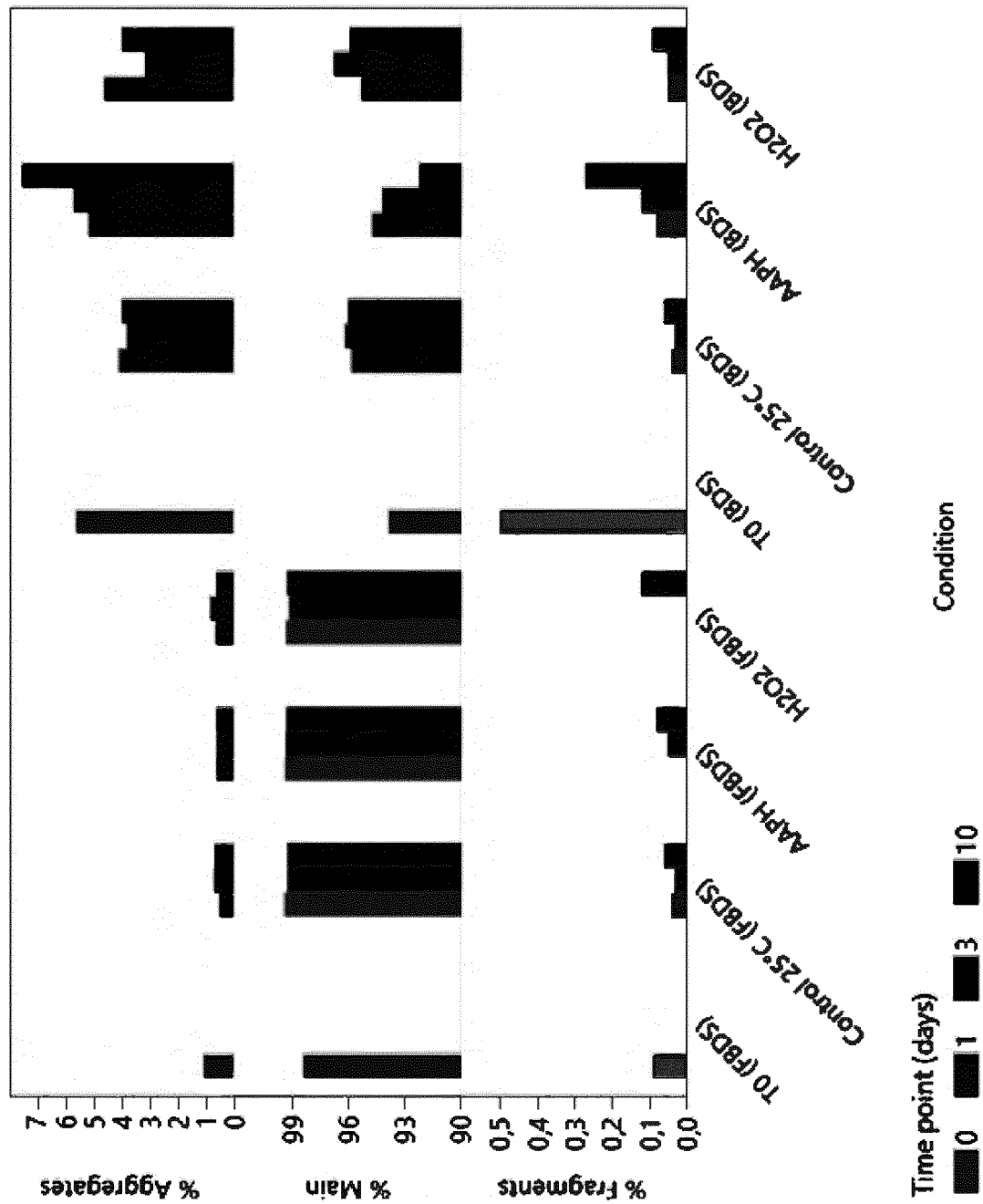

% Aggregates
% Main
% Fragments
0
1
2
3
4
5
6
7
90
93
96
99
0,0
0,1
0,2
0,3
0,4
0,5
T0 (FBDS)
Control 25°C (FBDS)
AAPH (FBDS)
H2O2 (FBDS)
T0 (BDS)
Control 25°C (BDS)
AAPH (BDS)
H2O2 (BDS)
Condition
Time point (days)
0
1
3
10

FIG. 6A

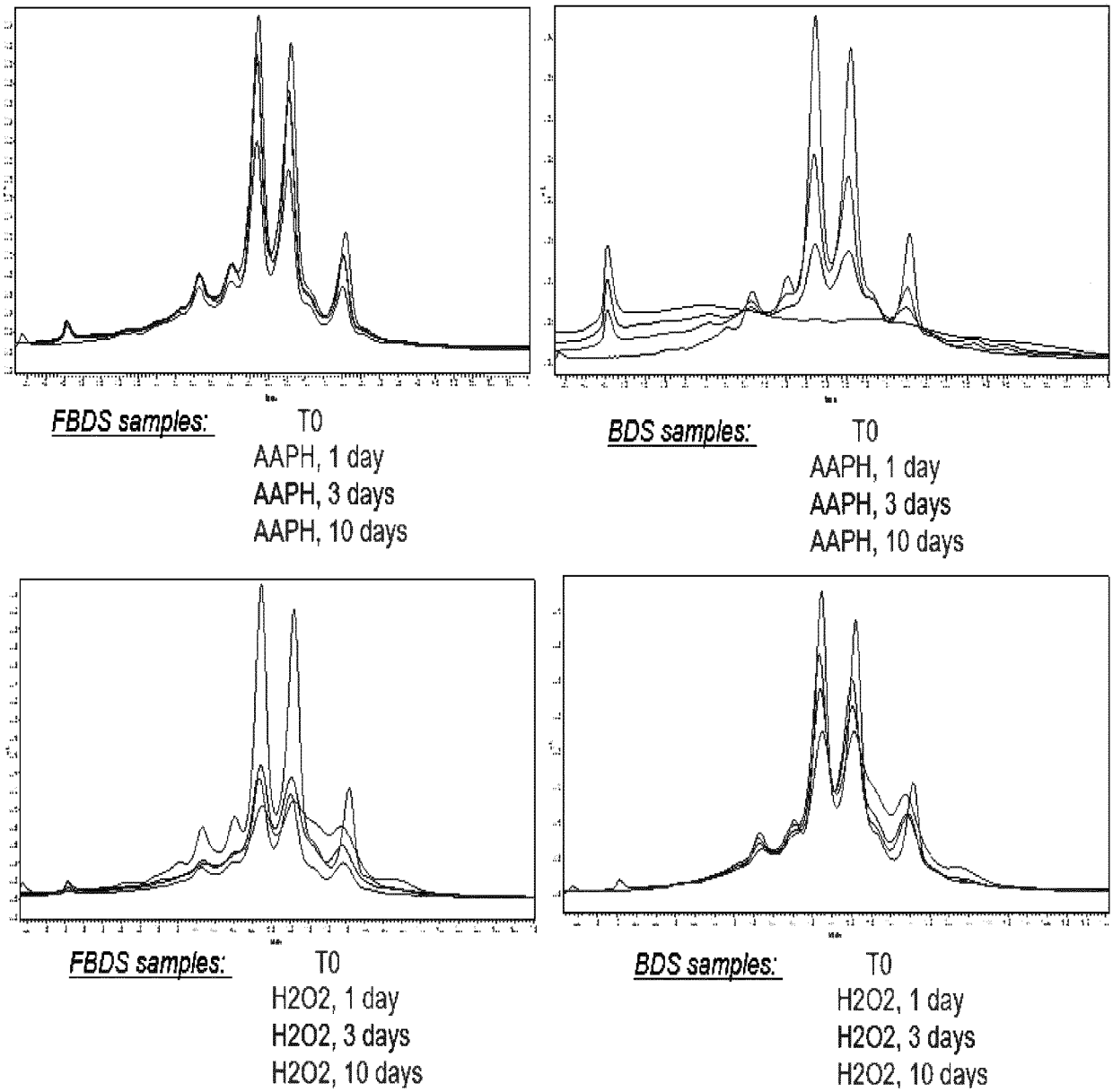

FBDS samples:
T0
AAPH, 1 day
AAPH, 3 days
AAPH, 10 days
BDS samples:
T0
AAPH, 1 day
AAPH, 3 days
AAPH, 10 days
FBDS samples:
T0
H2O2, 1 day
H2O2, 3 days
H2O2, 10 days
BDS samples:
T0
H2O2, 1 day
H2O2, 3 days
H2O2, 10 days

FIG. 6B

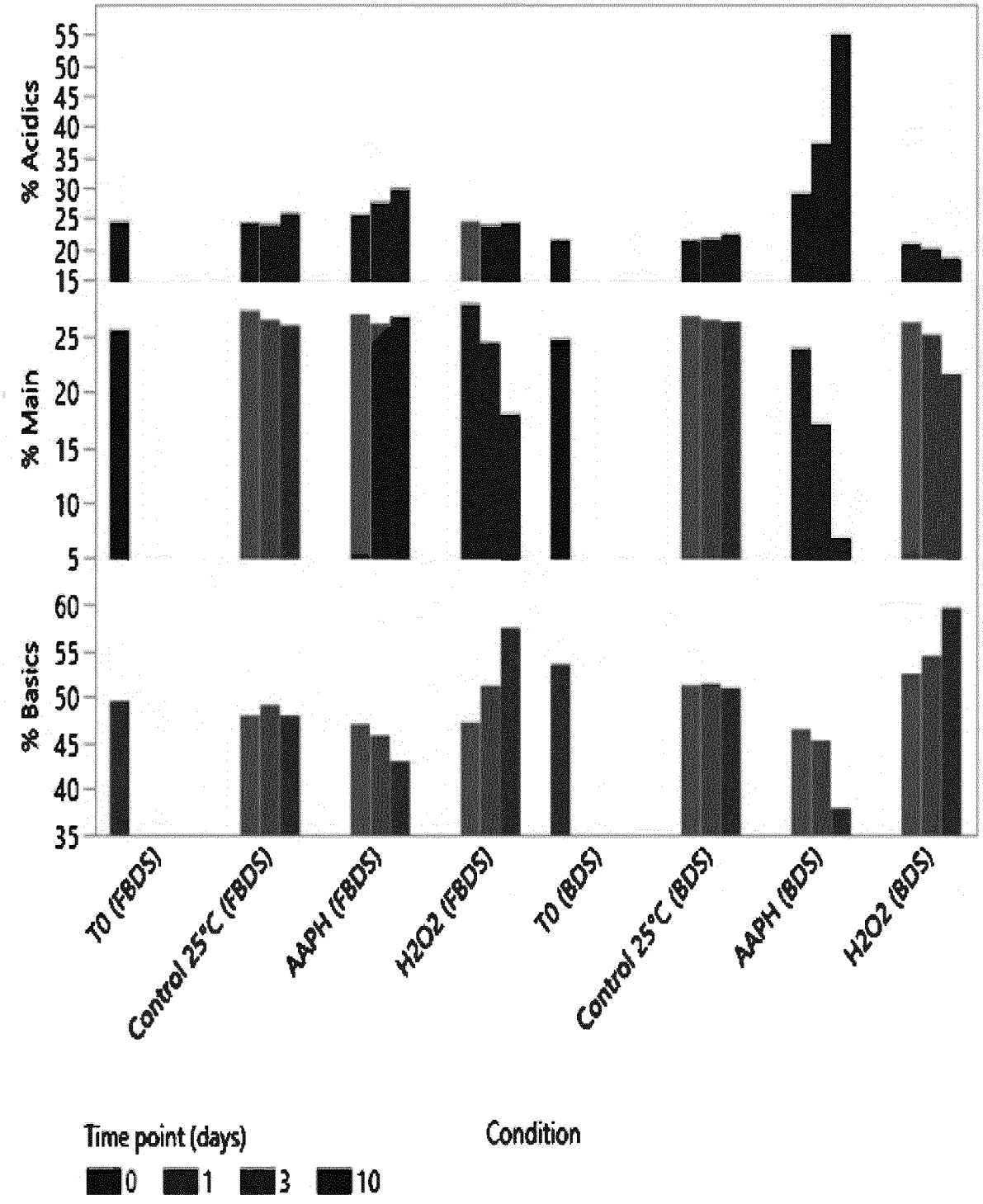

% Acidics
% Main
% Basics
T0 (FBDS)
Control 25°C (FBDS)
AAPH (FBDS)
H2O2 (FBDS)
T0 (BDS)
Control 25°C (BDS)
AAPH (BDS)
H2O2 (BDS)
Time point (days)
0
1
3
10
Condition

FIG. 7A

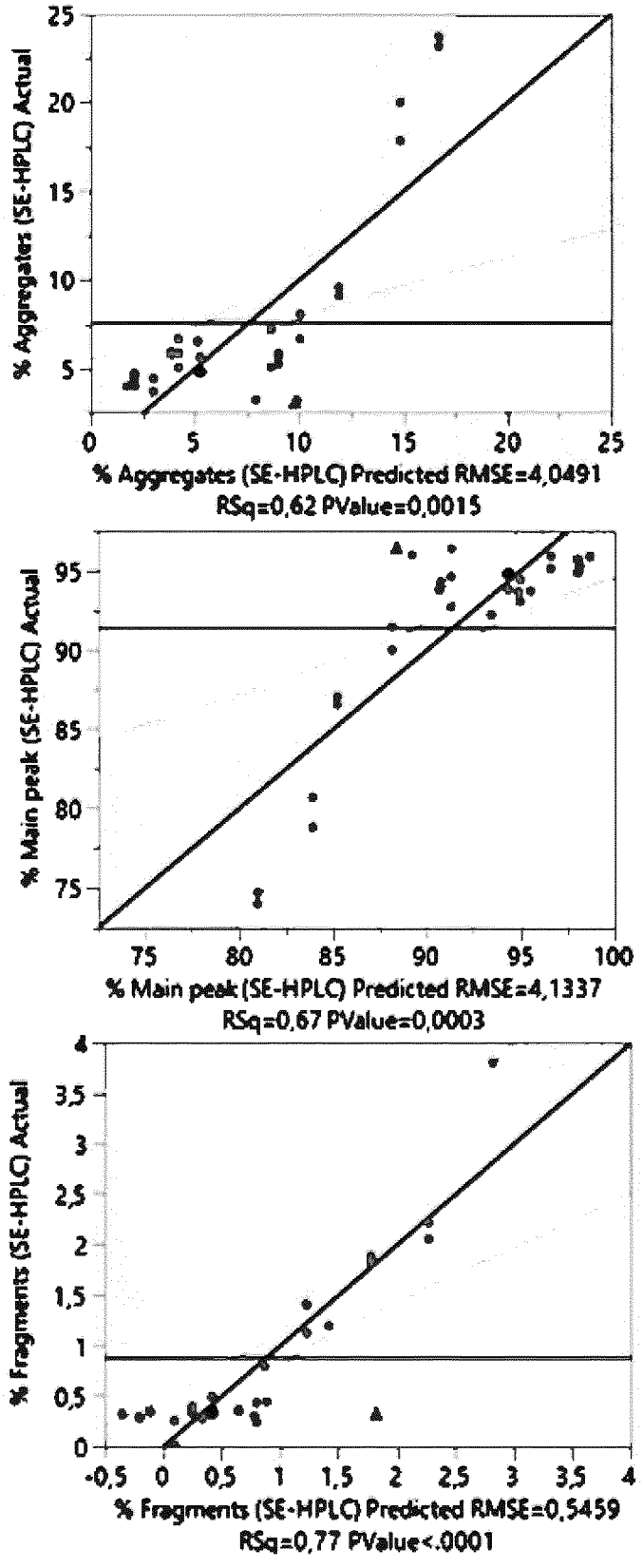

% Aggregates (SE-HPLC) Actual
% Aggregates (SE-HPLC) Predicted RMSE=4,0491
RSq=0,62 PValue=0,0015
% Main peak (SE-HPLC) Actual
% Main peak (SE-HPLC) Predicted RMSE=4,1337
RSq=0,67 PValue=0,0003
% Fragments (SE-HPLC) Actual
% Fragments (SE-HPLC) Predicted RMSE=0,5459
RSq=0,77 PValue<.0001

FIG. 7B

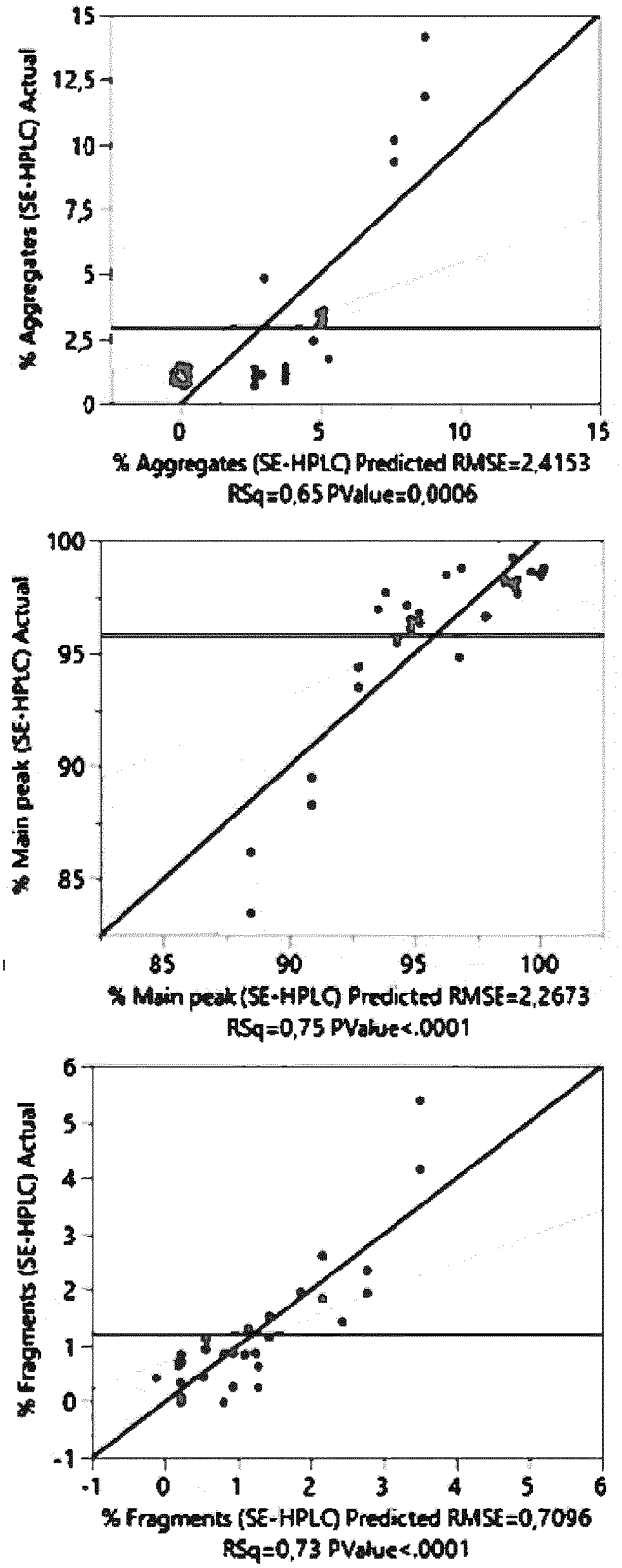

% Aggregates (SE-HPLC) Actual
15
12,5
10
7,5
5
2,5
0
0
5
10
15
% Aggregates (SE-HPLC) Predicted RMSE=2,4153
RSq=0,65 PValue=0,0006
% Main peak (SE-HPLC) Actual
100
95
90
85
85
90
95
100
% Main peak (SE-HPLC) Predicted RMSE=2,2673
RSq=0,75 PValue<.0001
% Fragments (SE-HPLC) Actual
6
5
4
3
2
1
0
-1
-1
0
1
2
3
4
5
6
% Fragments (SE-HPLC) Predicted RMSE=0,7096
RSq=0,73 PValue<.0001

FIG. 8

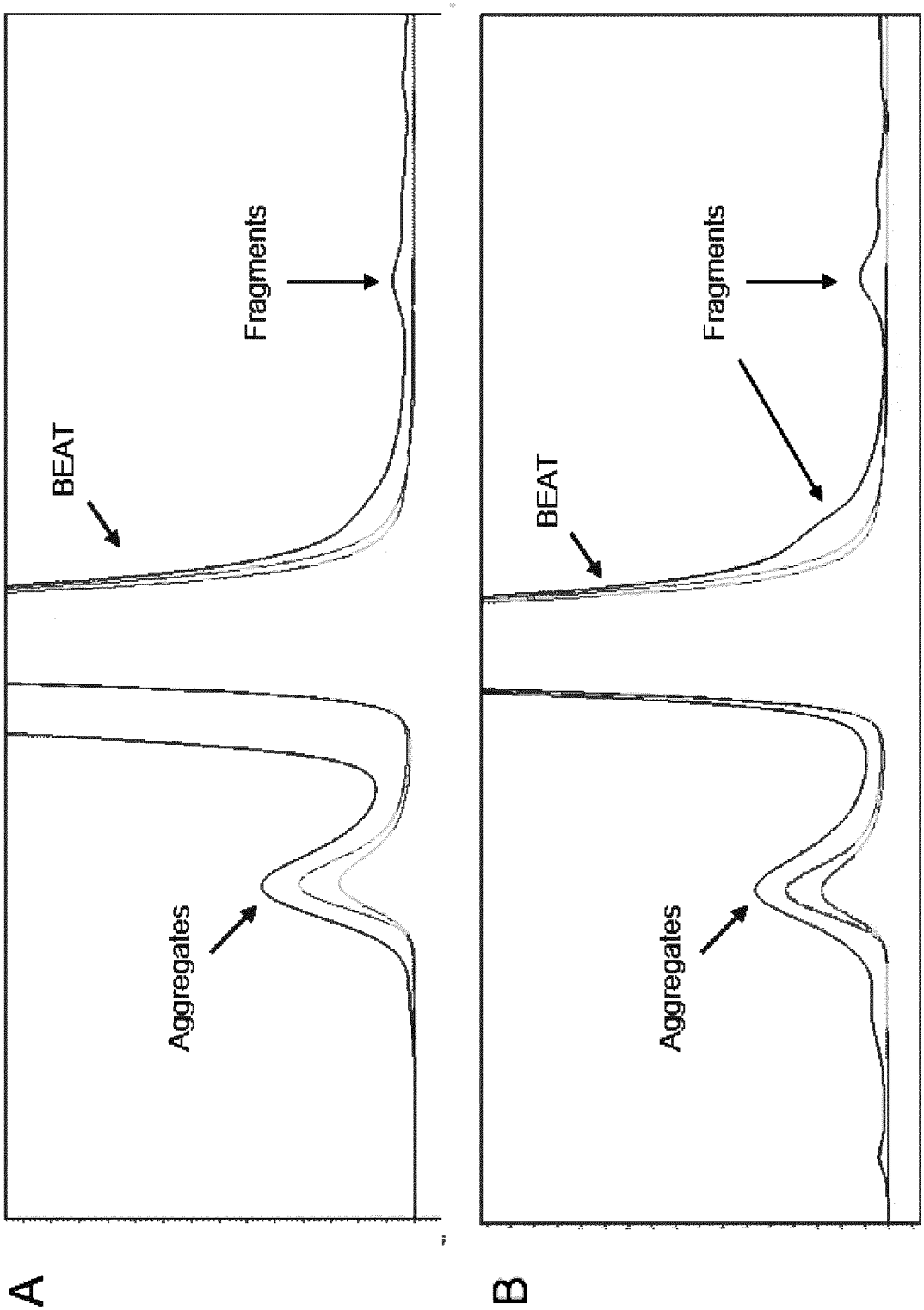


FIG. 9

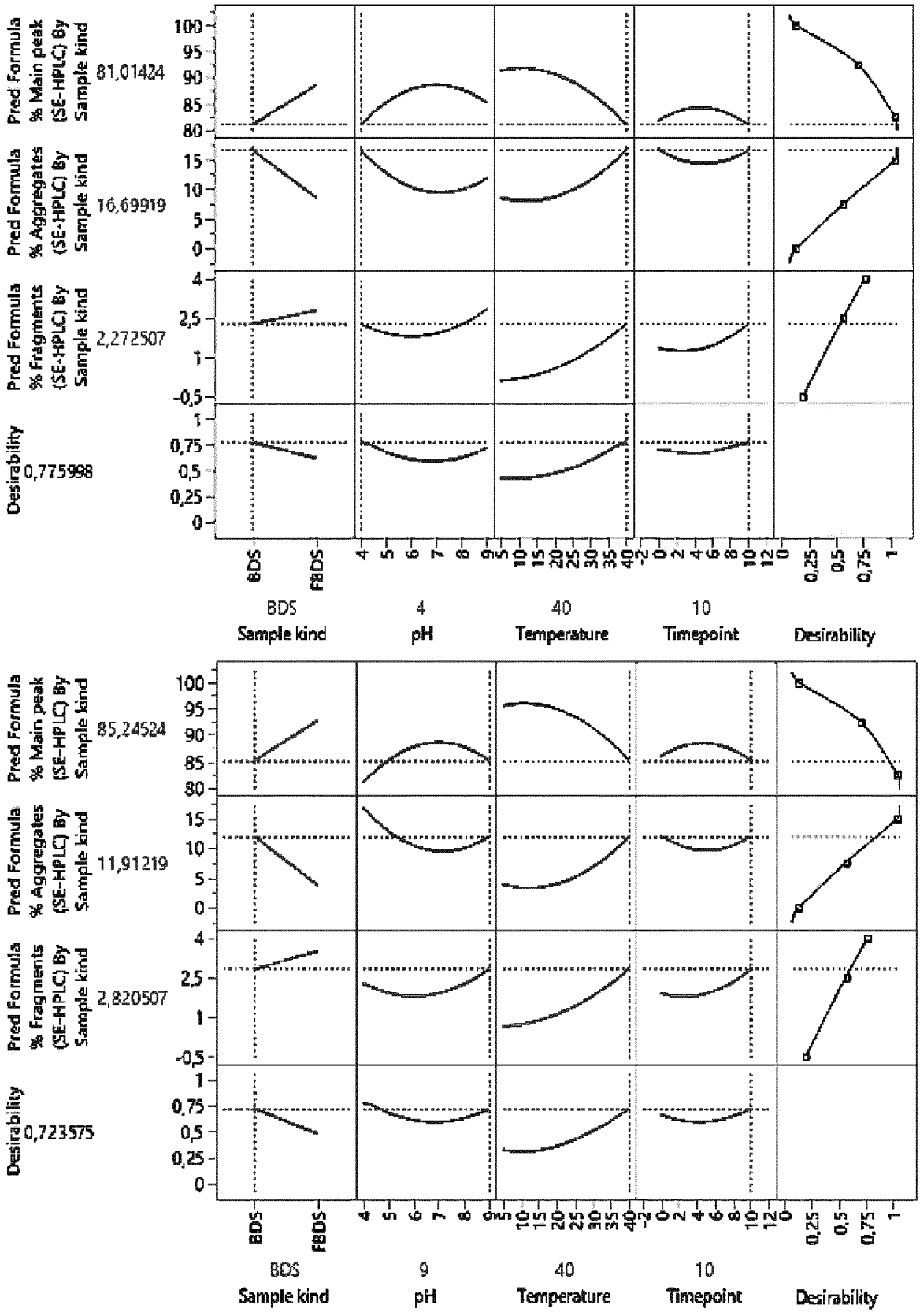

Pred Formula % Main peak (SE-HPLC) By Sample kind
81,01424
Pred Formula % Aggregates (SE-HPLC) By Sample kind
16,69919
Pred Formula % Fragments (SE-HPLC) By Sample kind
2,272507
Desirability
0,775998
BDS
FBDS
BDS
Sample kind
4
pH
40
Temperature
10
Timepoint
Desirability
Pred Formula % Main peak (SE-HPLC) By Sample kind
85,24524
Pred Formula % Aggregates (SE-HPLC) By Sample kind
11,91219
Pred Formula % Fragments (SE-HPLC) By Sample kind
2,820507
Desirability
0,723575
BDS
FBDS
BDS
Sample kind
9
pH
40
Temperature
10
Timepoint
Desirability

FIG. 10

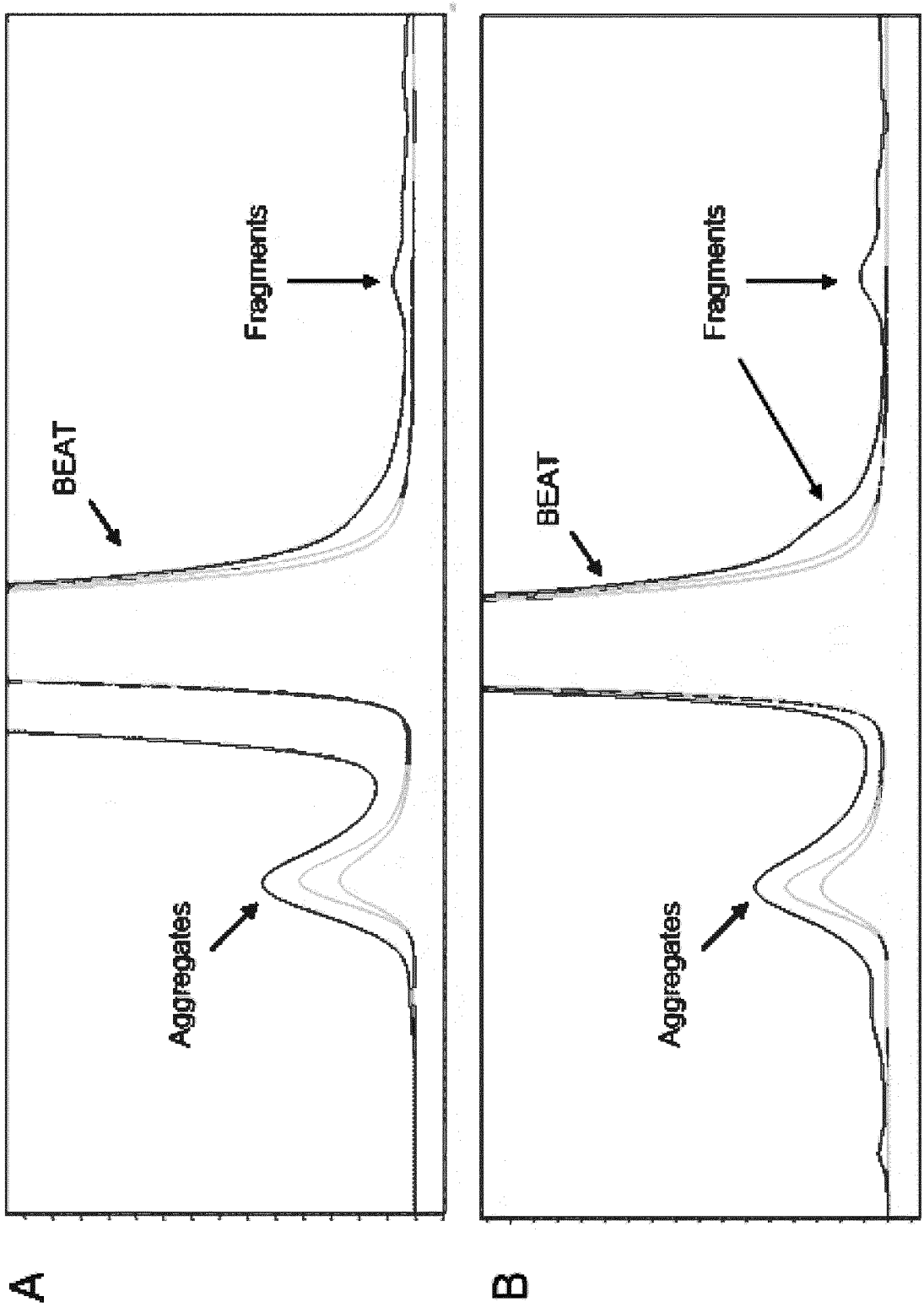


FIG. 11

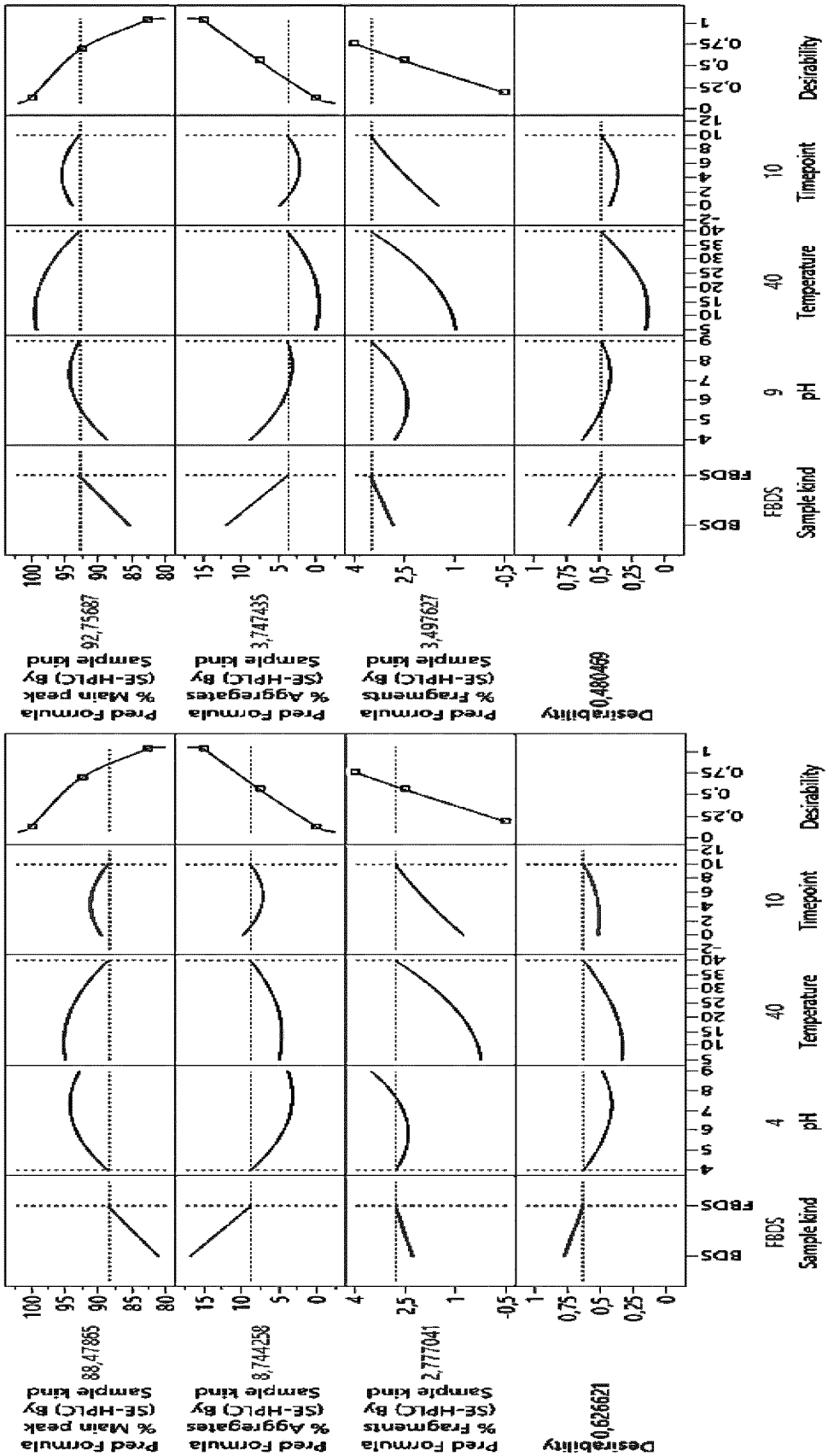

Pred Formula % Main peak (SE-HPLC) by Sample kind
92,75687
Pred Formula % Aggregates (SE-HPLC) by Sample kind
3,747435
Pred Formula % Fragments (SE-HPLC) by Sample kind
3,497627
Desirability
0,480469
Pred Formula % Main peak (SE-HPLC) by Sample kind
88,47865
Pred Formula % Aggregates (SE-HPLC) by Sample kind
8,744258
Pred Formula % Fragments (SE-HPLC) by Sample kind
2,777041
Desirability
0,626621
Sample kind
BDS
FBDS
pH
Temperature
Timepoint
Desirability

FIG. 12

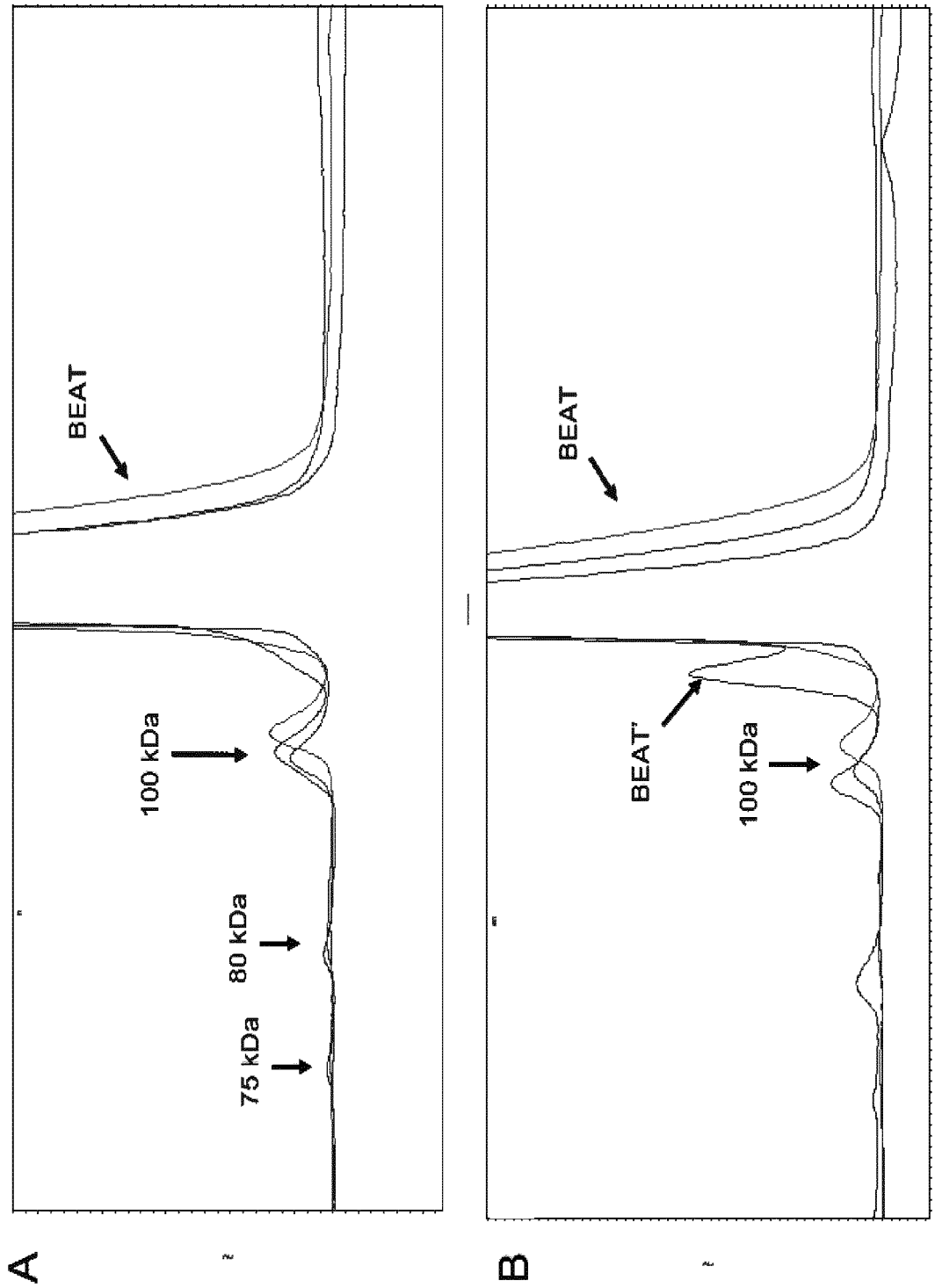


FIG. 13

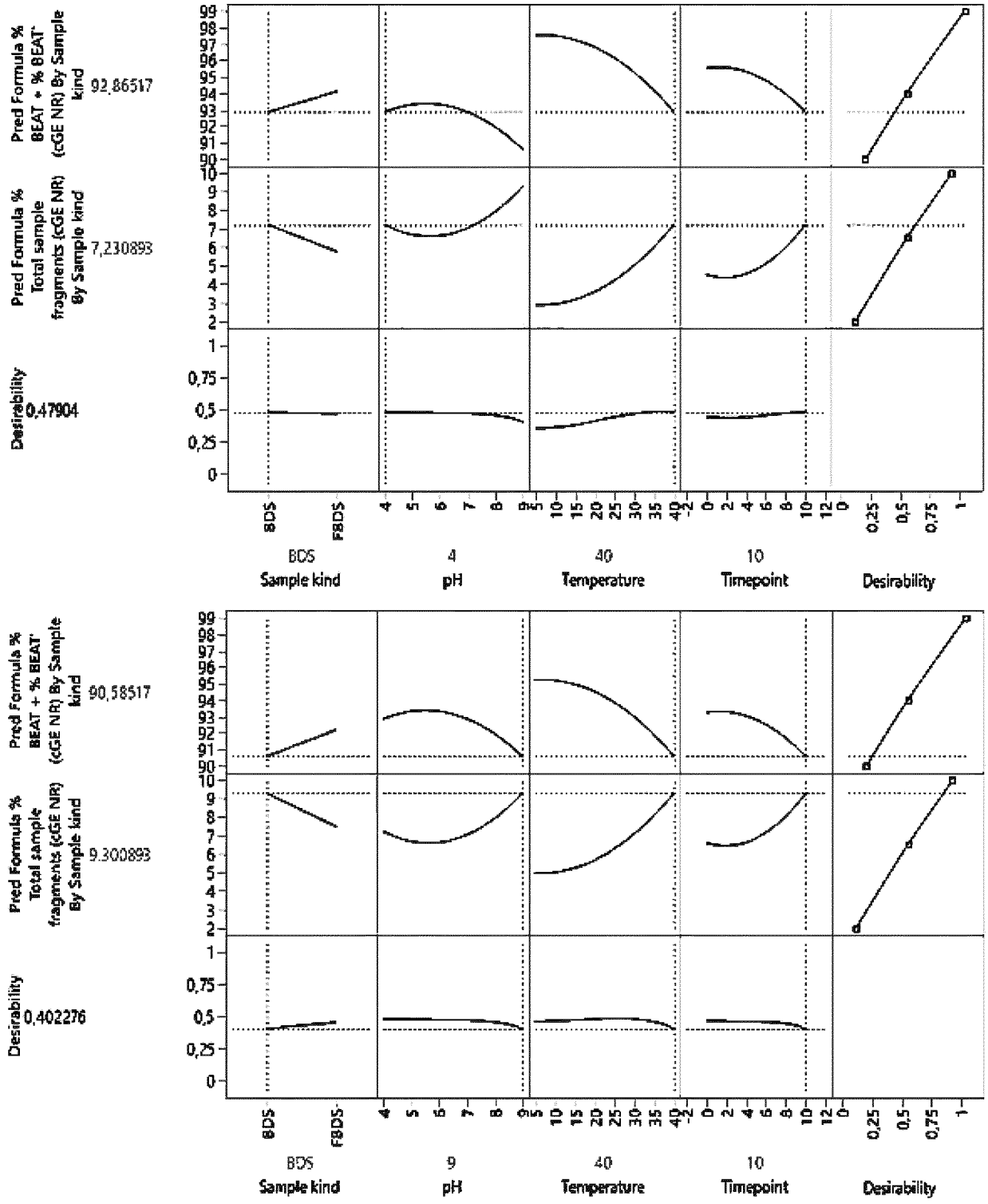

Pred Formula % BEAT + % BEAT (cGE NR) By Sample kind
92,86517
Pred Formula % Total sample fragments (cGE NR) By Sample kind
7,230893
Desirability
0,47904
BDS
FBDS
4
40
10
Sample kind
pH
Temperature
Timepoint
Desirability
Pred Formula % BEAT + % BEAT (cGE NR) By Sample kind
90,58517
Pred Formula % Total sample fragments (cGE NR) By Sample kind
9,300893
Desirability
0,402276
BDS
FBDS
9
40
10
Sample kind
pH
Temperature
Timepoint
Desirability

FIG. 14

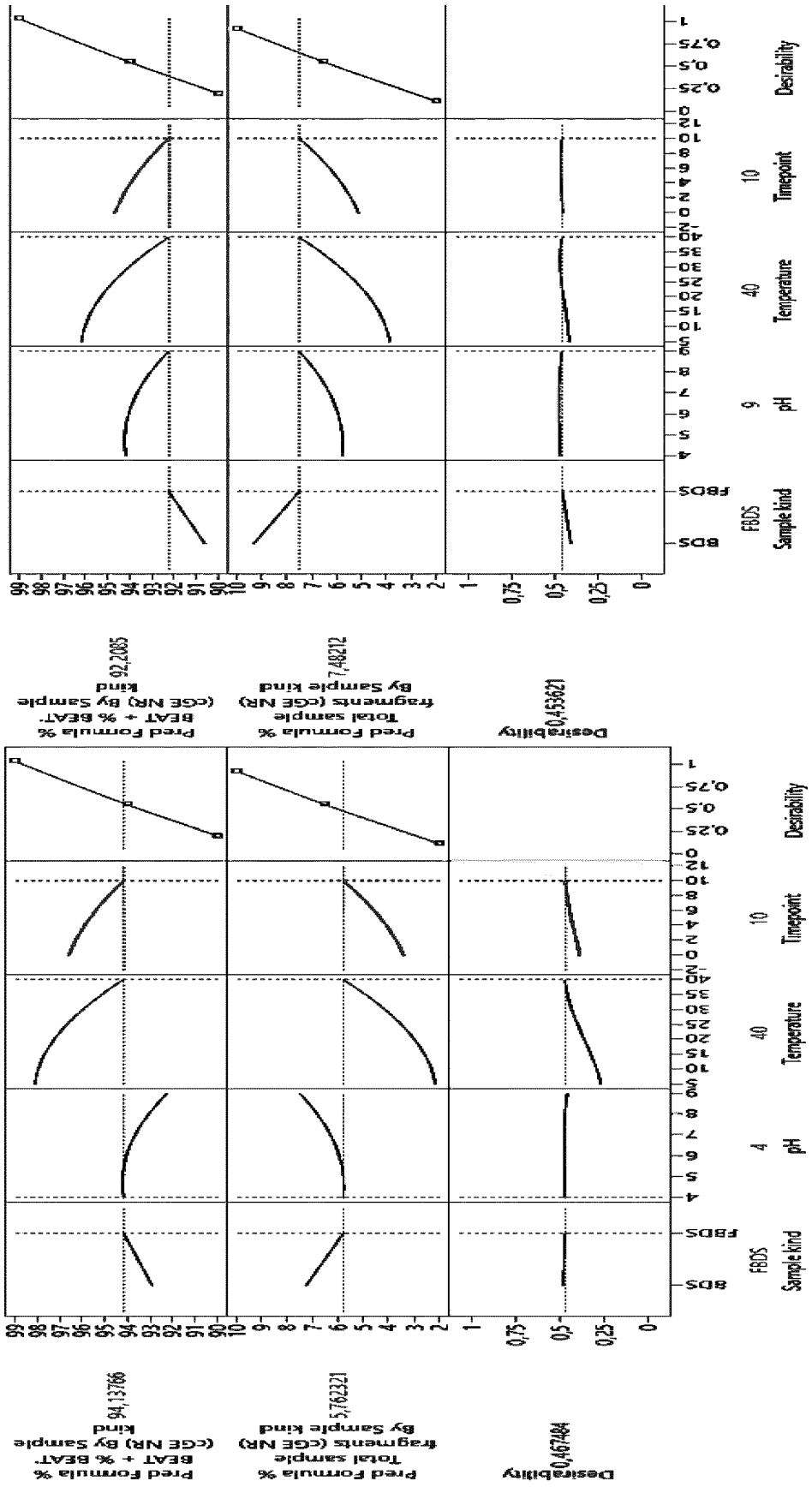

Pred Formula % BEAT + % BEAT (cGE NR) By Sample kind
94,13766
Pred Formula % Total sample fragments (cGE NR) By Sample kind
5,762321
Desirability
0,467484
Pred Formula % BEAT + % BEAT (cGE NR) By Sample kind
92,2085
Pred Formula % Total sample fragments (cGE NR) By Sample kind
7,48212
Desirability
0,453621
Sample kind
pH
Temperature
Timepoint
Desirability
FBDS
BDS

FIG. 15

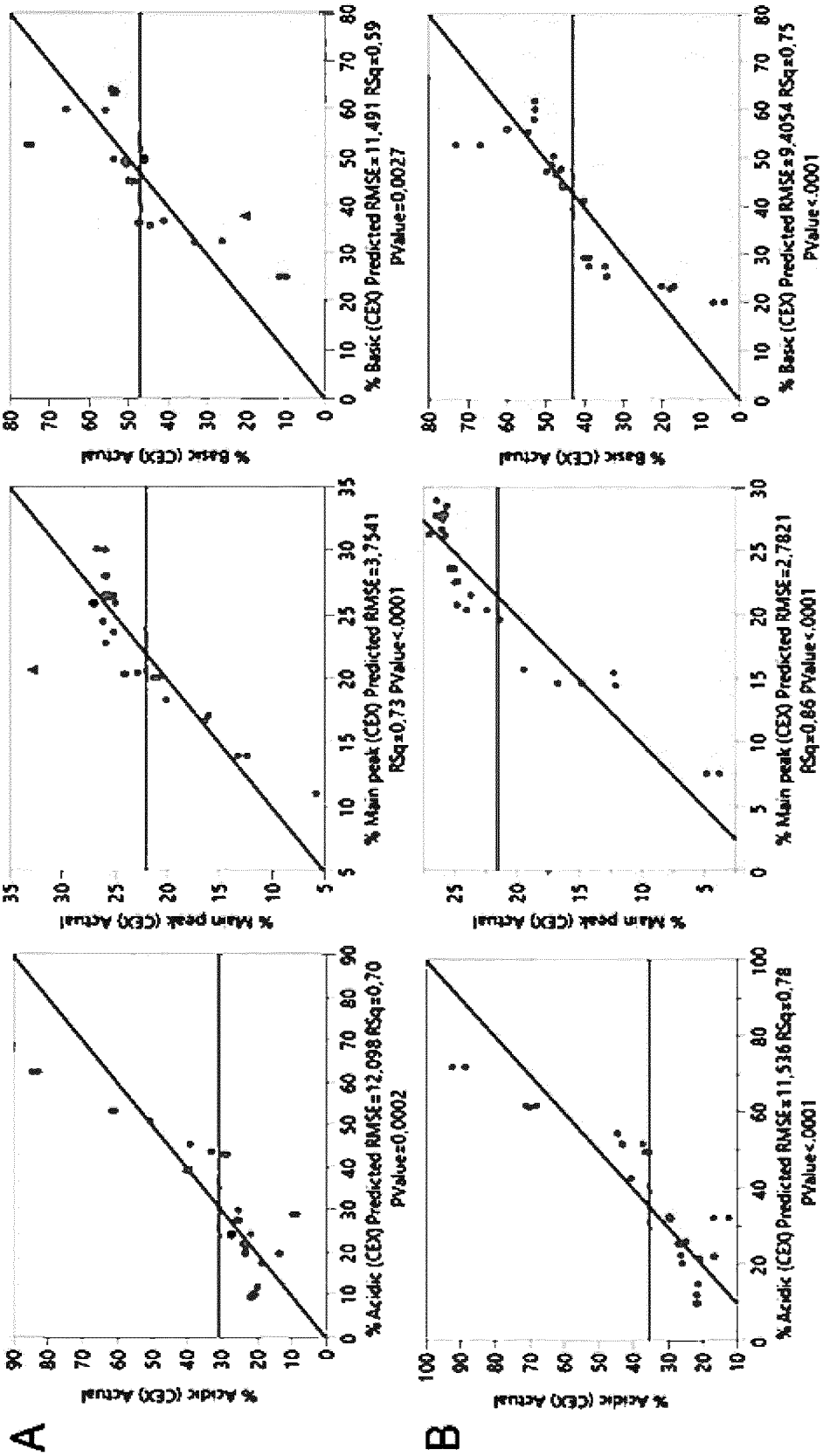

A
% Acidic (CEX) Actual
% Acidic (CEX) Predicted RMSE=12,098 RSq=0,70
PValue=0,0002
% Main peak (CEX) Actual
% Main peak (CEX) Predicted RMSE=3,7341
RSq=0,73 PValue<,0001
% Basic (CEX) Actual
% Basic (CEX) Predicted RMSE=11,491 RSq=0,59
PValue=0,0027
B
% Acidic (CEX) Actual
% Acidic (CEX) Predicted RMSE=11,536 RSq=0,78
PValue<,0001
% Main peak (CEX) Actual
% Main peak (CEX) Predicted RMSE=2,7821
RSq=0,86 PValue<,0001
% Basic (CEX) Actual
% Basic (CEX) Predicted RMSE=9,4054 RSq=0,75
PValue<,0001

FIG. 16

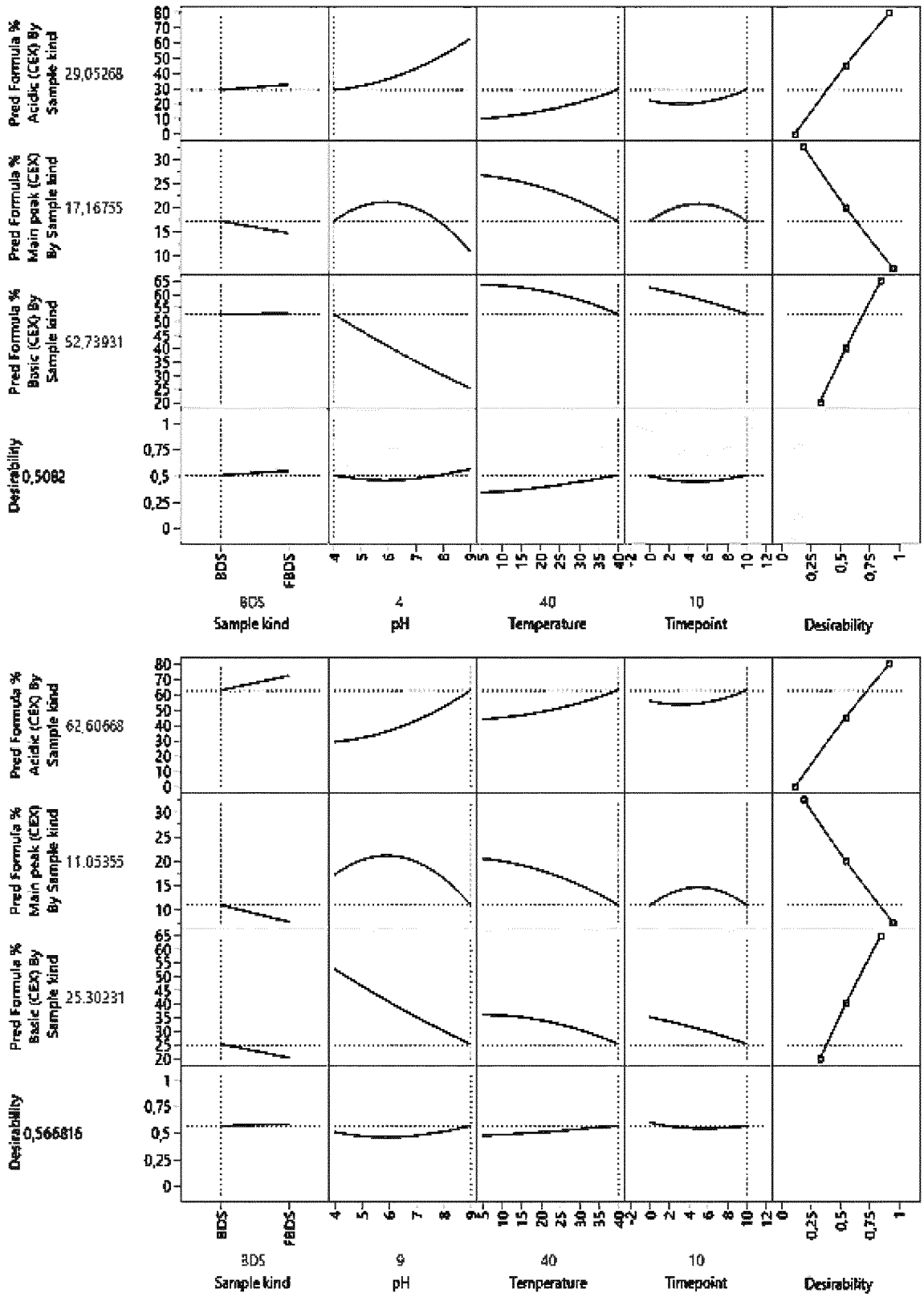

Pred Formula % Acidic (CEX) By Sample kind
29,05268
Pred Formula % Main peak (CEX) By Sample kind
17,16755
Pred Formula % Basic (CEX) By Sample kind
52,73931
Desirability
0,5082
BDS
FBDS
BDS
Sample kind
4
pH
40
Temperature
10
Timepoint
Desirability
Pred Formula % Acidic (CEX) By Sample kind
62,60668
Pred Formula % Main peak (CEX) By Sample kind
11,05355
Pred Formula % Basic (CEX) By Sample kind
25,30231
Desirability
0,566816
BDS
FBDS
BDS
Sample kind
9
pH
40
Temperature
10
Timepoint
Desirability

FIG. 17

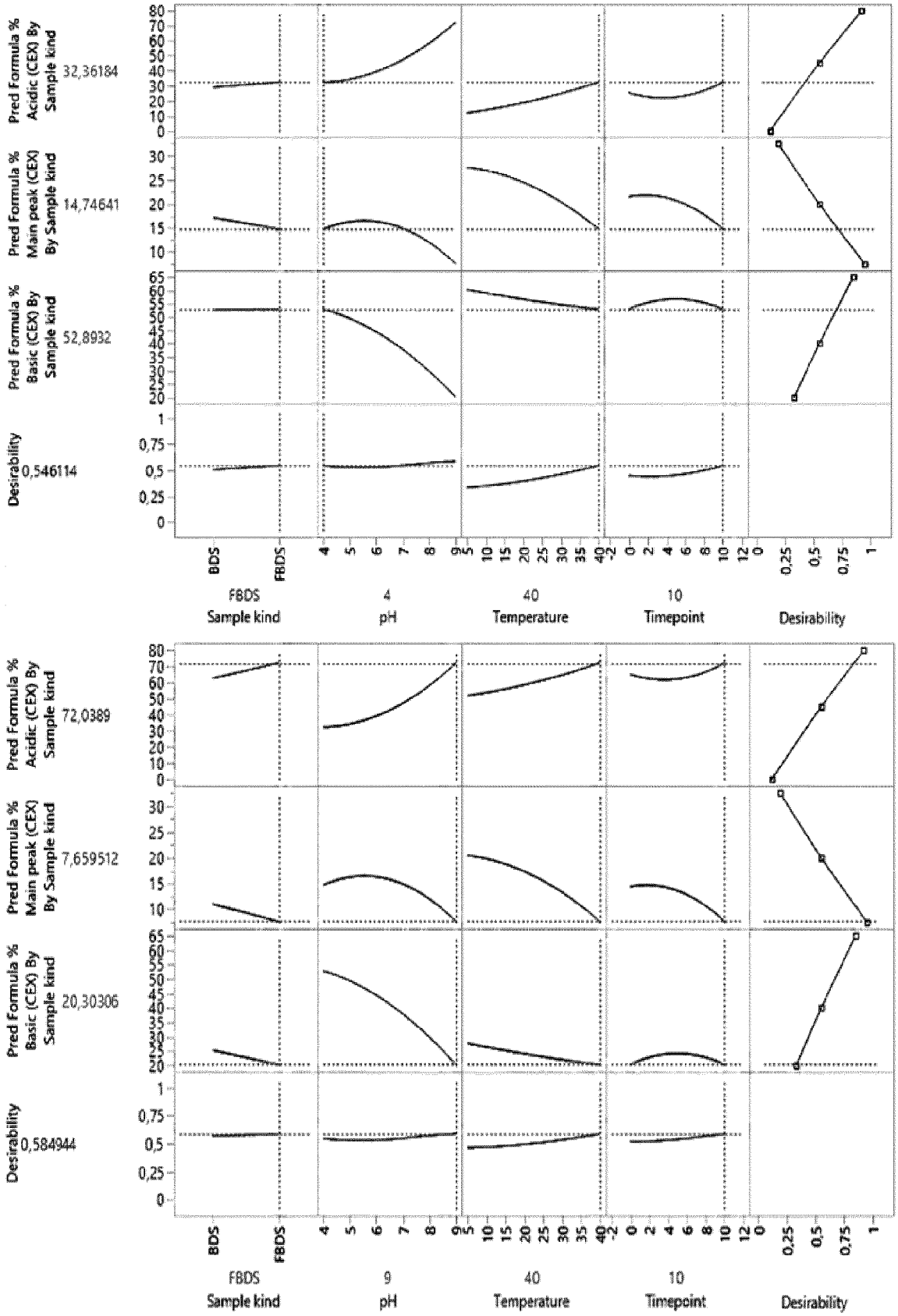

Pred Formula % Acidic (CEX) By Sample kind
32,36184
Pred Formula % Main peak (CEX) By Sample kind
14,74641
Pred Formula % Basic (CEX) By Sample kind
52,8932
Desirability
0,546114
FBDS
Sample kind
4
pH
40
Temperature
10
Timepoint
Desirability
Pred Formula % Acidic (CEX) By Sample kind
72,0389
Pred Formula % Main peak (CEX) By Sample kind
7,659512
Pred Formula % Basic (CEX) By Sample kind
20,30306
Desirability
0,584944
FBDS
Sample kind
9
pH
40
Temperature
10
Timepoint
Desirability

FIG. 18

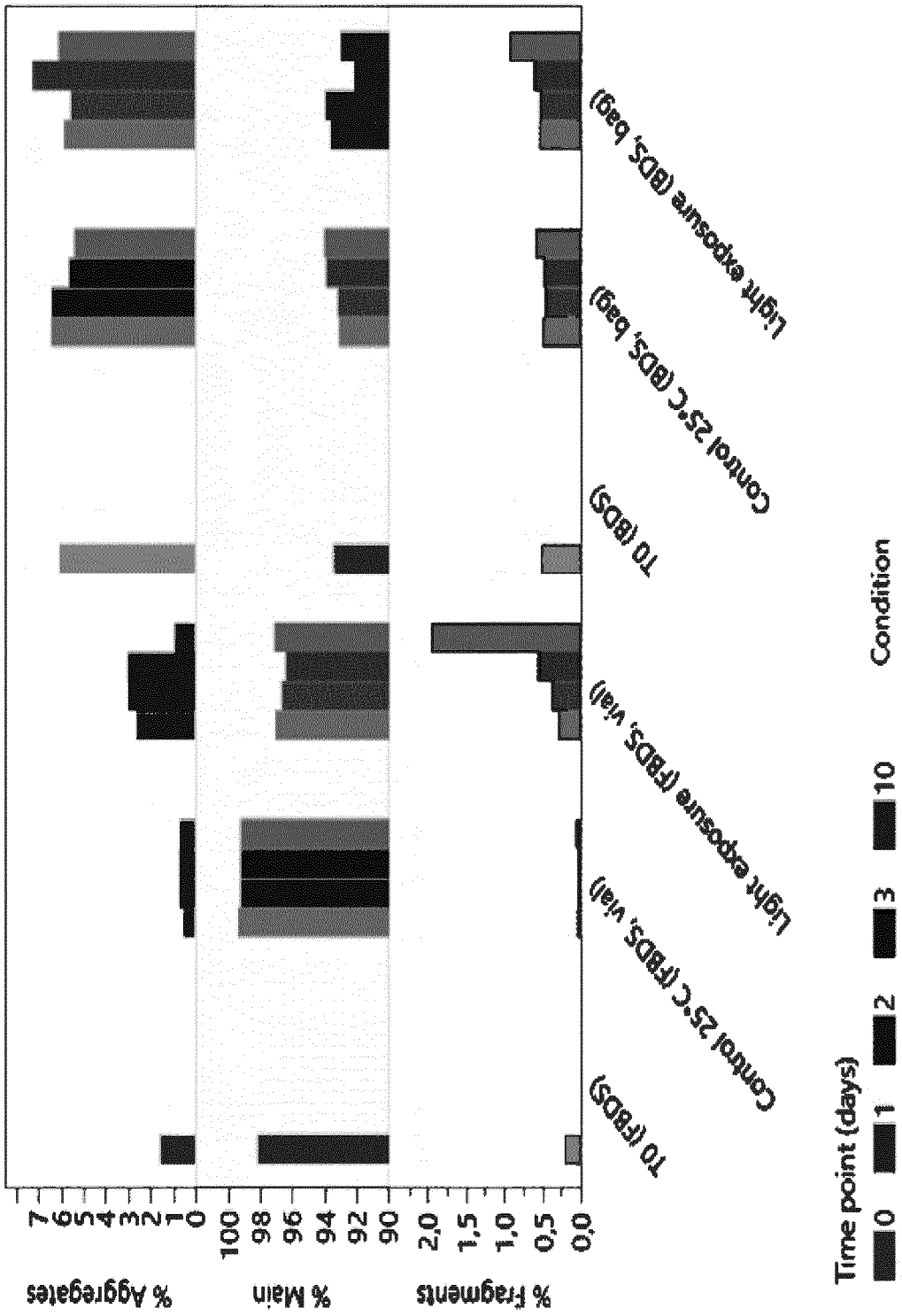

% Aggregates
% Main
% Fragments
T0 (FBDS)
Control 25°C (FBDS, vial)
Light exposure (FBDS, vial)
T0 (BDS)
Control 25°C (BDS, bag)
Light exposure (BDS, bag)
Time point (days)
0
1
2
3
10
Condition

FIG. 19

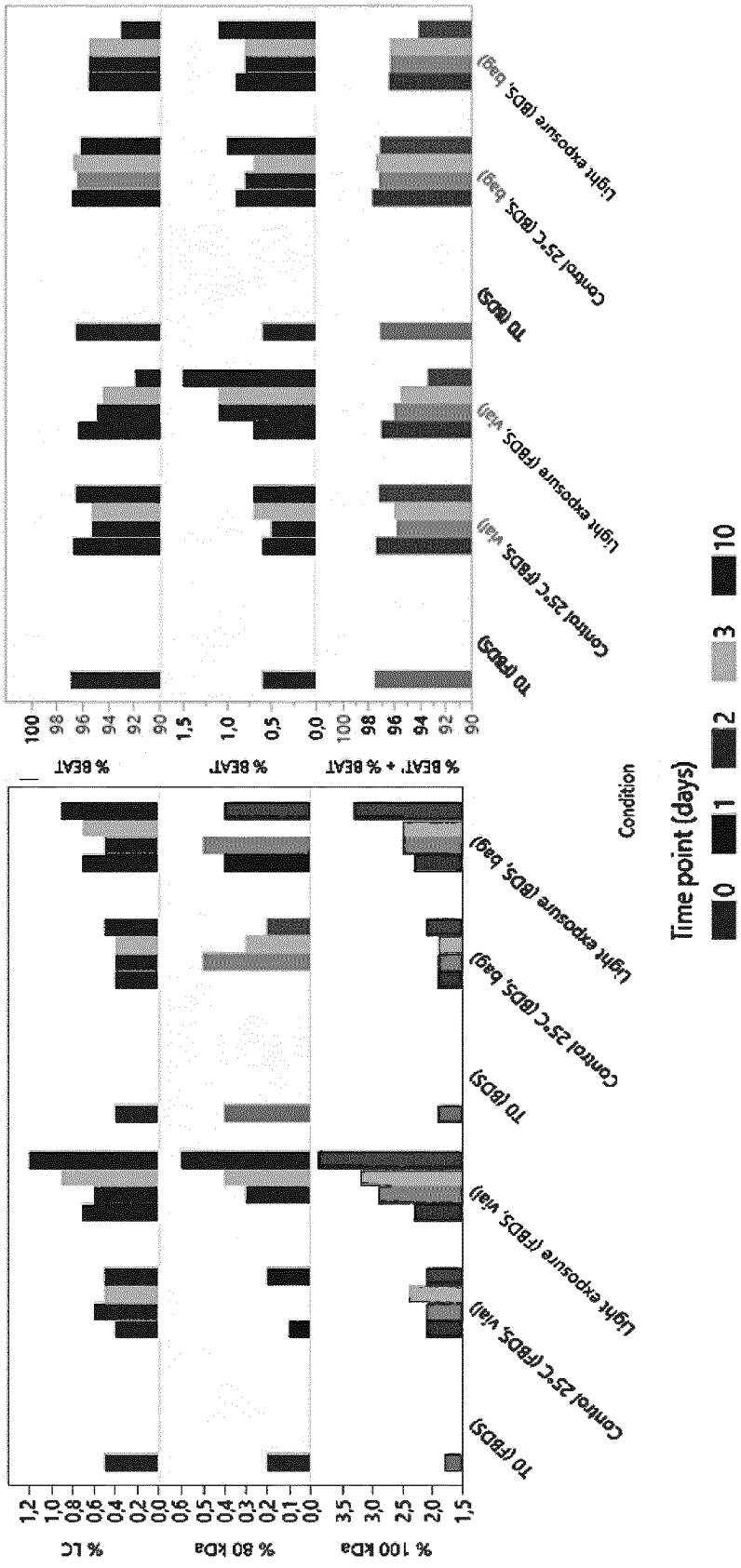

% 1C
% 80 kDa
% 100 kDa
% BEAT
% BEAT
% BEAT' + % BEAT
T0 (FBDS)
Control 25°C (FBDS, vial)
Light exposure (FBDS, vial)
T0 (BDS)
Control 25°C (BDS, bag)
Light exposure (BDS, bag)
Condition
Time point (days)
0
1
2
3
10

FIG. 20

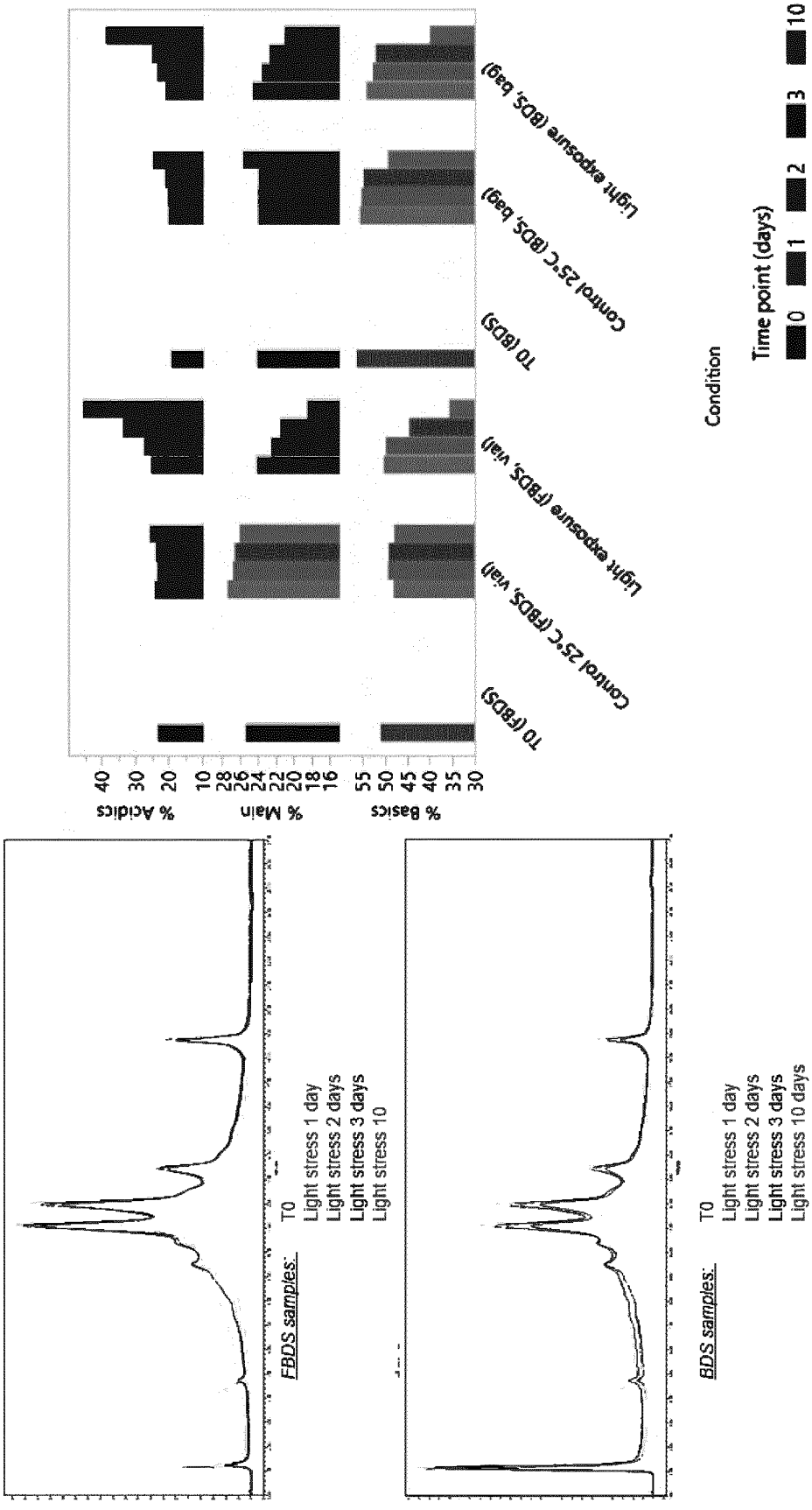

FBDS samples:
T0
Light stress 1 day
Light stress 2 days
Light stress 3 days
Light stress 10
BDS samples:
T0
Light stress 1 day
Light stress 2 days
Light stress 3 days
Light stress 10 days
% Acidics
% Main
% Basics
T0 (FBDS)
Control 25°C (FBDS, vial)
Light exposure (FBDS, vial)
T0 (BDS)
Control 25°C (BDS, bag)
Light exposure (BDS, bag)
Condition
Time point (days)
0
1
2
3
10

FIG. 21

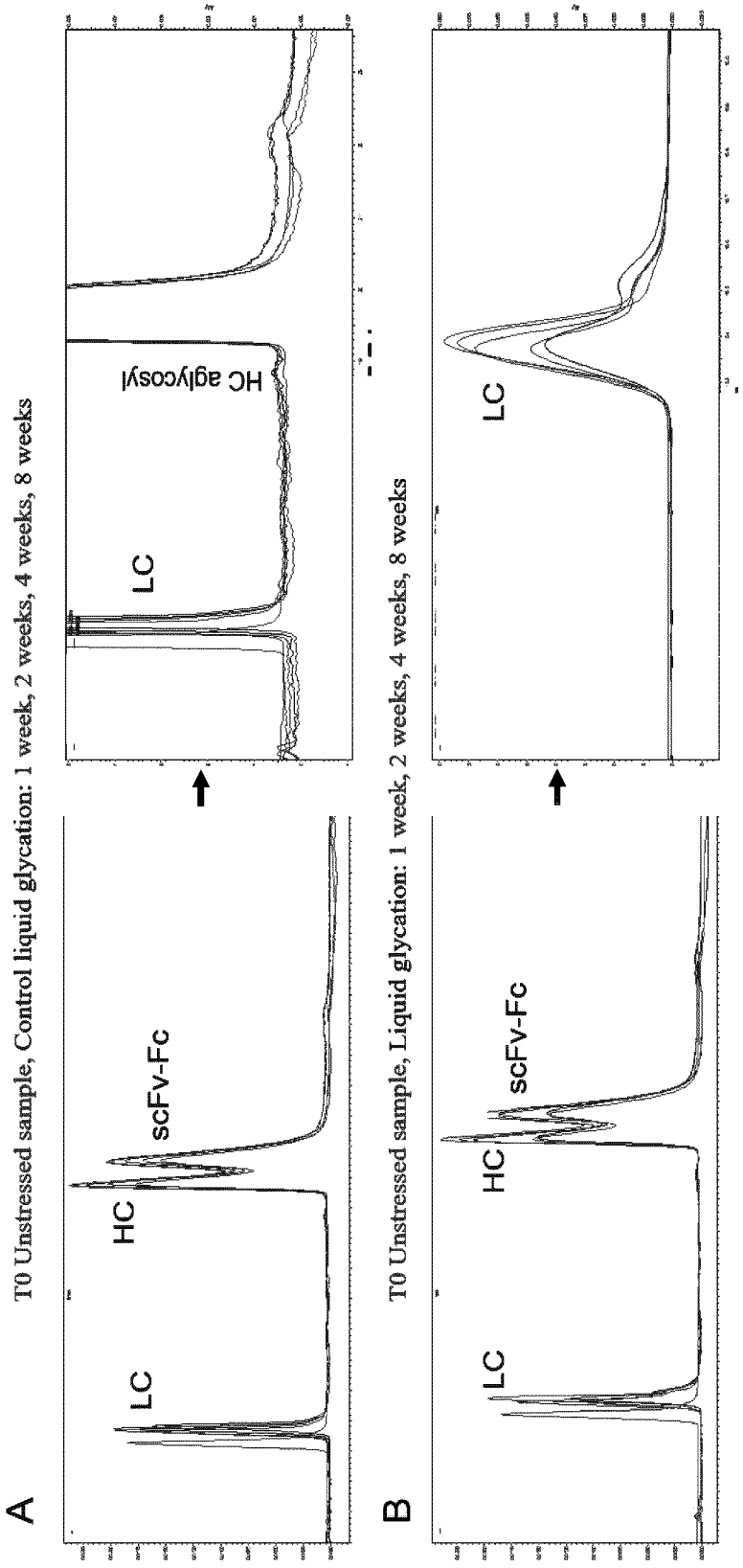

A
T0 Unstressed sample, Control liquid glycation: 1 week, 2 weeks, 4 weeks, 8 weeks
LC
HC
scFv-Fc
LC
HC aglycosyl
B
T0 Unstressed sample, Liquid glycation: 1 week, 2 weeks, 4 weeks, 8 weeks
LC
HC
scFv-Fc
LC

FIG. 22A

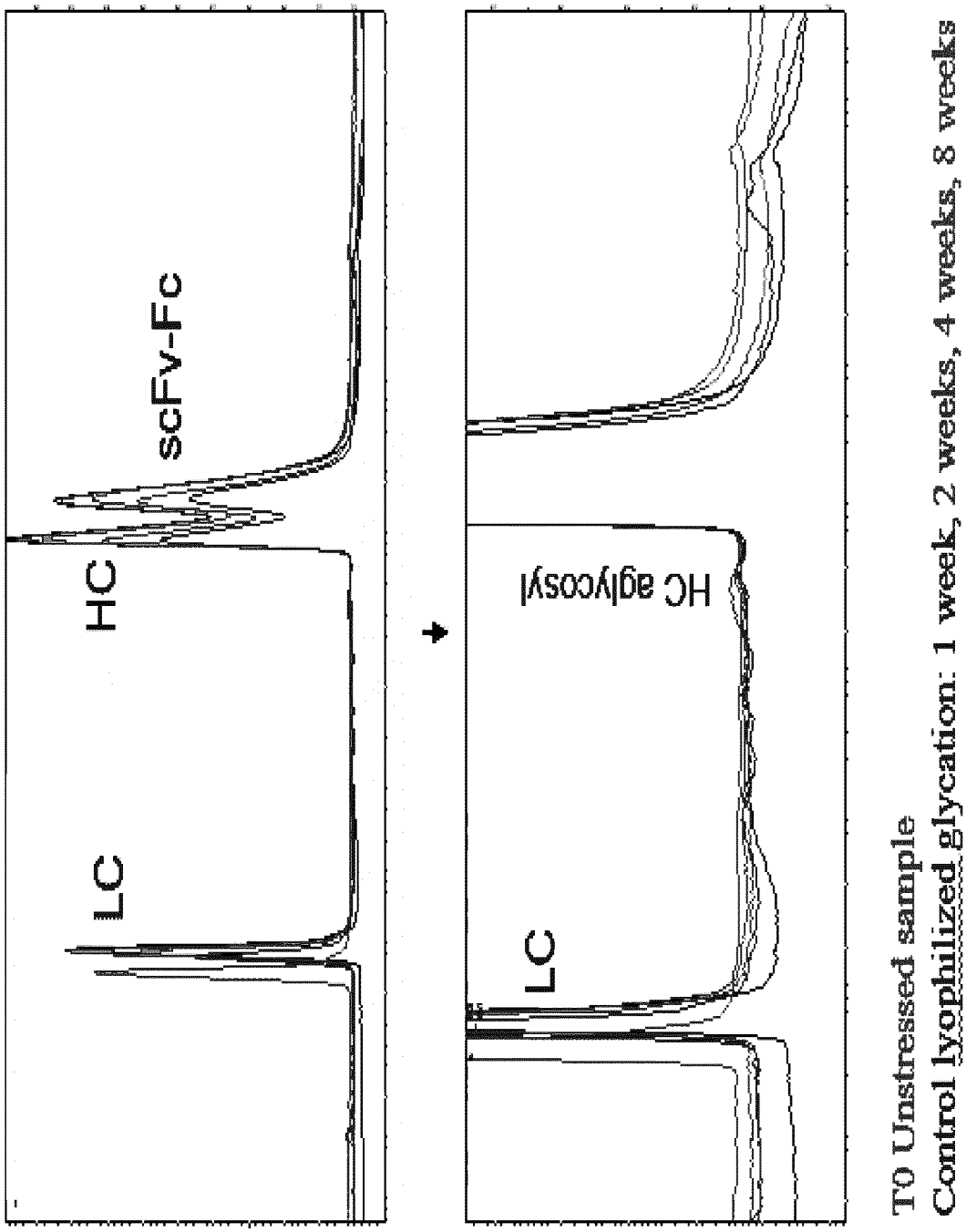
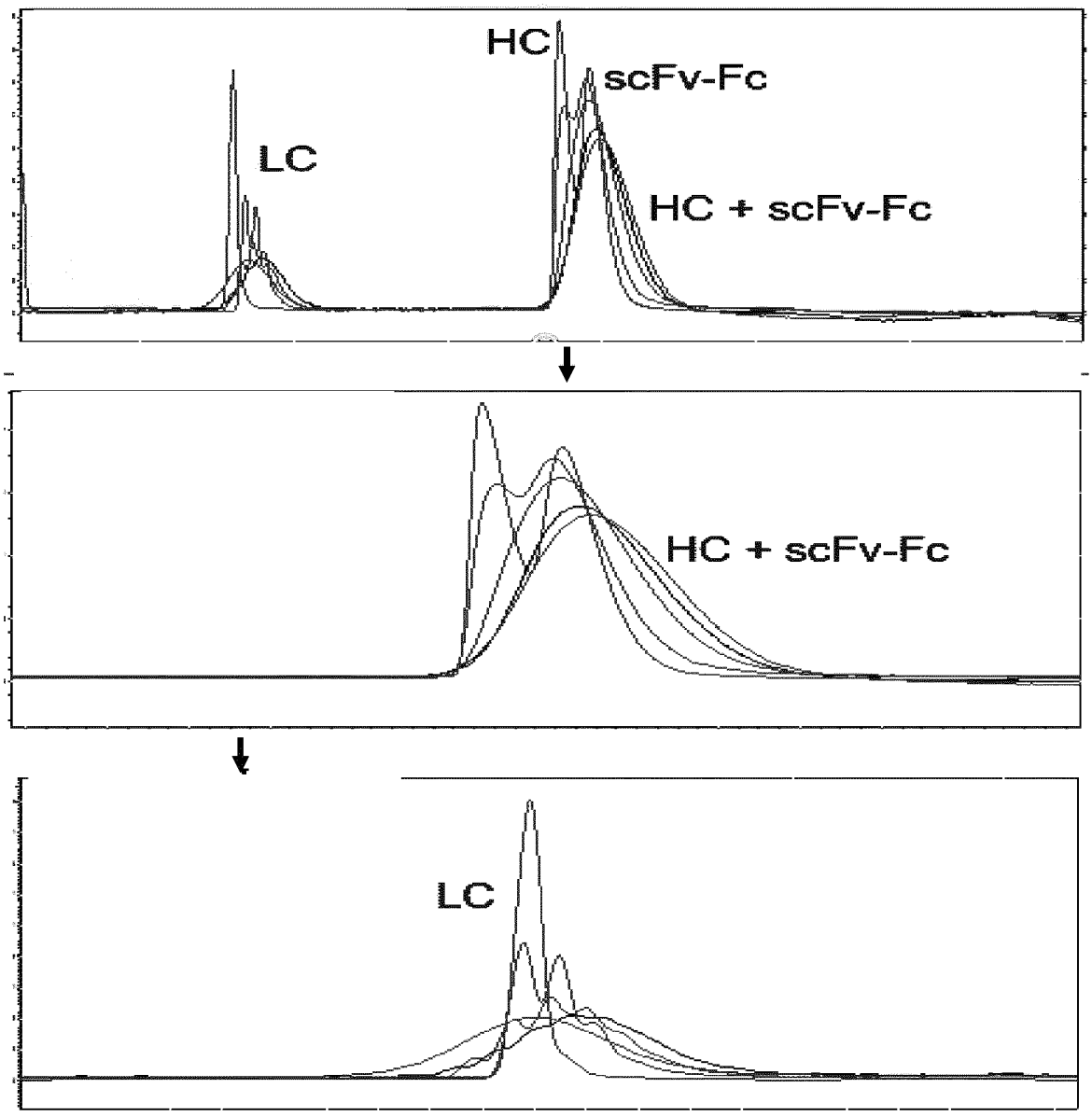

LC
HC
scFv-Fc
LC
HC aglycosyl
T0 Unstressed sample
Control lyophilized glycation: 1 week, 2 weeks, 4 weeks, 8 weeks

FIG. 22B



T0 Unstressed sample
Lyophilized glycation: 1 week, 2 weeks, 4 weeks, 8 weeks

FIG. 23

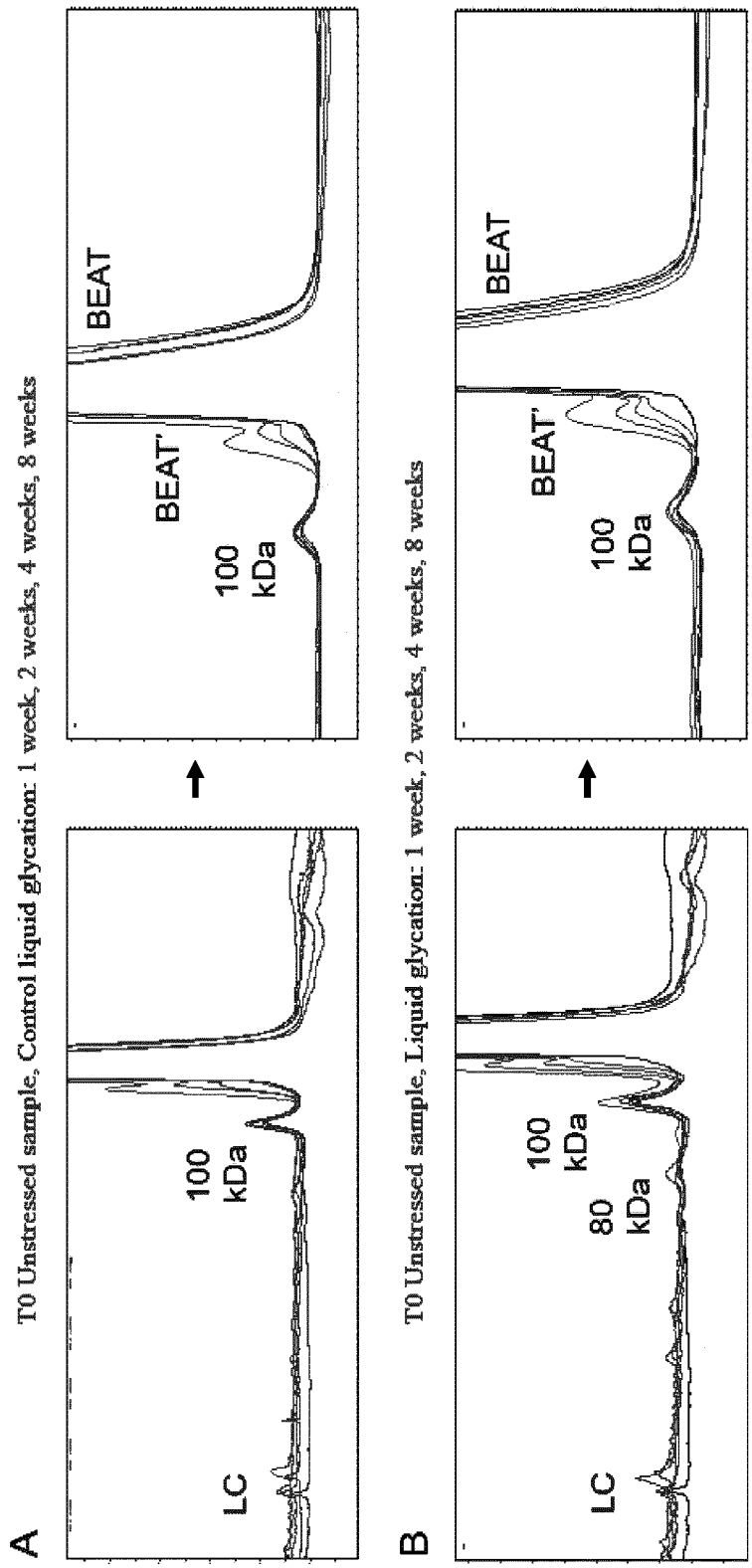
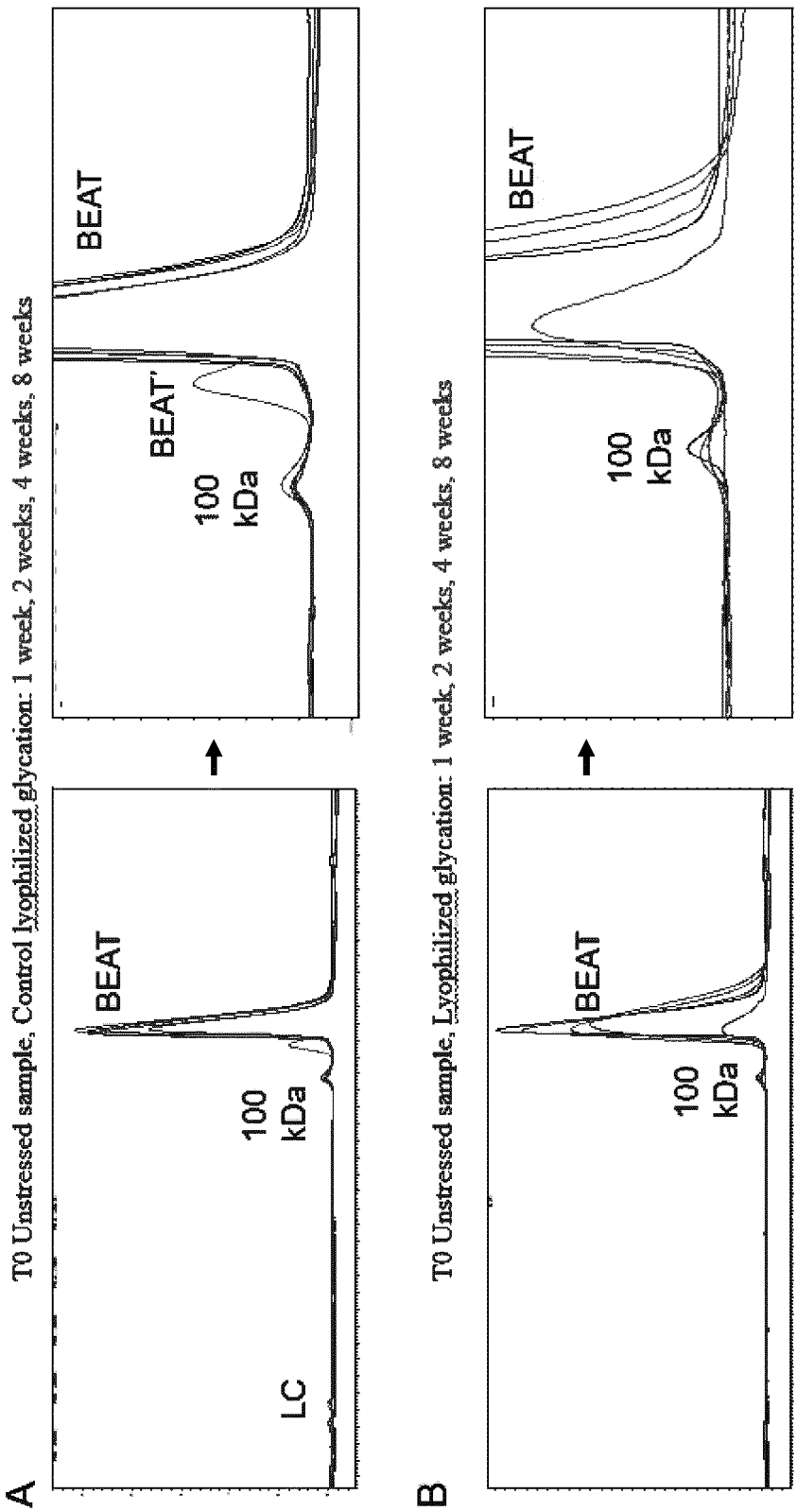

A
T0 Unstressed sample, Control liquid glycation: 1 week, 2 weeks, 4 weeks, 8 weeks
LC
100 kDa
BEAT'
BEAT
B
T0 Unstressed sample, Liquid glycation: 1 week, 2 weeks, 4 weeks, 8 weeks
LC
100 kDa
80 kDa
BEAT'
BEAT

FIG. 24


A
T0 Unstressed sample, Control lyophilized glycation: 1 week, 2 weeks, 4 weeks, 8 weeks
BEAT
100 kDa
LC
BEAT
BEAT'
100 kDa
B
T0 Unstressed sample, Lyophilized glycation: 1 week, 2 weeks, 4 weeks, 8 weeks
BEAT
100 kDa
BEAT
100 kDa

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2019/074270

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K9/00 A61K39/00 A61K9/08 A61K9/19 A61K47/18
A61K47/26
ADD.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

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

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2009/070642 A1 (MEDIMMUNE LLC [US]; SHARMA MONIKA S [US] ET AL.) 4 June 2009 (2009-06-04) paragraph [0698] - paragraph [0820] claims 1-63 -----	1-19
X	WO 2016/036678 A1 (MEDIMMUNE LLC [US]) 10 March 2016 (2016-03-10) claims 1-22 paragraph [0066] - paragraph [0111] -----	1-19
X	WO 2017/188356 A1 (CHUGAI PHARMACEUTICAL CO LTD [JP]) 2 November 2017 (2017-11-02) claims 1-13 paragraph [0002] - paragraph [0061] -/--	1-19

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
17 December 2019	22/01/2020

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Schifferer, Hermann

3

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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X	EP 2 982 692 A1 (ENGMAB AG [CH]) 10 February 2016 (2016-02-10) example 9 -----	1-19
X	WO 2014/177568 A1 (SANOFI SA [FR]) 6 November 2014 (2014-11-06) claims 1-40 -----	1-19

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International application No
PCT/EP2019/074270

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2019/074270

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		TW 201534323 A	16-09-2015
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		WO 2014177568 A1	06-11-2014