STABILIZED GLYCEROL FREE PHARMACEUTICAL FORMULATIONS OF INSULIN ANALOGUES AND/OR INSULIN DERIVATIVES

INTRODUCTION

The present invention relates to a pharmaceutical formulation of at least one insulin analogue and/or insulin derivative, a process for preparing the pharmaceutical formulation of at least on insulin analogue and/or insulin derivative, and a related kit. It also relates to the pharmaceutical formulation of at least one insulin analogue and/or insulin derivative and to the related kit for use in the treatment of diabetes mellitus, hyperglycemia, and/or for use in lowering blood glucose levels. The present invention also relates to the use of a medical device for administering the pharmaceutical formulation of at least one insulin analogue and/or insulin derivative to an animal and/or human.

BACKGROUND OF THE INVENTION

Diabetes mellitus is a metabolic disorder in which the ability to utilize glucose is more or less completely lost.

For decades, insulin has been used in the treatment of diabetes mellitus. Several insulin formulations have been developed, e.g. insulin zinc suspension (containing Zn(II)), formulations containing protamine, etc. Further, the active pharmaceutical ingredient insulin itself has been modified by developing fast acting insulin analogues (e.g. insulin aspart, insulin lispro, insulin glulisine) and long acting insulin analogues and derivatives (e.g. insulin detemir, insulin degludec, insulin glargin). Fast acting insulin preparations are usually solutions of insulin, while long acting insulin preparations can be suspensions containing insulin in crystalline and/or amorphous form precipitated by the addition of zinc (Zn(II)) salts alone or by addition of protamine or by a combination of both.

The chemical and physical stability of insulin formulations is very important. Insulin formulations are often administered by using pen injection devices or insulin pumps in which an insulin formulation is stored in cartridges until the entire cartridge is empty. Insulin formulations may also be stored in vials, requiring a stable formulation with respect to chemical and physical stability across the shelf life of the formulation.

The chemical and/or physical stability of insulin, insulin analogues and/or insulin derivatives strongly depends on the pharmaceutical formulation, e.g. the solvent, the pH value and the excipients. Brange et al. (Acta Pharm. Nord. 4(3), pp. 149-158, 1992) disclose several aspects in connection with the chemical stability of insulin. WO 2004/080480 discloses pharmaceutical preparations comprising acid-stabilized insulin. GB 835,638 discloses insulin crystal suspensions having a protracted effect. WO 98/56406 discloses stable insulin formulations. US 6,489,292 discloses stable aqueous insulin preparations without phenol and cresol. US 6,211,144 discloses stable concentrated insulin preparations for pulmonary delivery. Bhatt et al. (Pharmaceutical Research, Vol. 7, No. 6, pp. 593-599, 1990) disclose chemical pathways of peptide degradation. Patel et al. (Pharmaceutical Research, Vol. 7, No. 7, pp. 703-711, 1990) disclose chemical pathways of peptide degradation. Tyler-Cross et al. (The Journal of Biological Chemistry, Vol. 266, No. 33, Issue of November 25, pp. 22549-22556, 1991) disclose effects of amino acid sequence, buffers, and ionic strength on the rate and mechanism of deamidation of asparagine residues in small peptides. GB 840,870 discloses improvements in or relating to insulin preparations. US 6,852,694 discloses stabilized insulin formulations. Galloway et al. (Diabetes – The Journal of the American Diabetes Association, Vol. 21, No. Suppl.2, pp. 637-648, 1972) disclose new forms of insulin. Jackson et al. disclose several aspects with regard to neutral regular insulin (Diabetes - The Journal of the American Diabetes Association, Vol. 21, No. 4, pp. 235-245, 1972). Lill (Pharmazie in unserer Zeit, No. 1, pp. 56-61, 2001) discloses general aspects in connection with insulin formulations. The German product specification of the medicinal product Berlinsulin® H Normal 3mL Pen discloses a formulation containing human insulin, metacresol, glycerol, water and optionally hydrochloric acid and sodium hydroxide for pH adjustment. The German product specification of the medicinal product Actrapid® discloses a formulation containing human insulin, zinc chloride, glycerol, metacresol, water and optionally sodium hydroxide and/or hydrochloric acid for pH adjustment. The FDA label of the medicinal product Lantus® discloses a formulation containing insulin glargine, zinc, m-cresol, glycerol 85 %, polysorbate 20 and water for injection, wherein the pH is adjusted to approximately 4 by addition of aqueous solutions of hydrochloric acid and/or sodium hydroxide. The FDA label of the medicinal product Humalog® discloses a formulation containing insulin lispro, glycerin, dibasic sodium phosphate, metacresol, zinc oxide, phenol and water for injection, wherein the pH is adjusted to 7.0-7.8 by addition of aqueous solutions of hydrochloric acid and/or sodium hydroxide. The FDA label of the medicinal product Apidra® discloses a formulation containing insulin glulisine, metacresol, tromethamine, sodium chloride, polysorbate 20 and water for injection, wherein the pH is adjusted to 7.0-7.8 by addition of aqueous solutions of hydrochlorid acid and/or sodium hydroxide.

The solubility of insulin, insulin analogues and/or insulin derivatives in aqueous media depends on the pH value. For example, the lowest solubility is shown close to the isoelectric point which is for human insulin around pH 5.3 and 5.4. Very good solubility can be observed at pH values below 4 and above 7. However, insulin suffers from degradation at strong acidic conditions and strong alkaline conditions. Therefore, most of the medicinal products containing insulin, insulin analogues and/or insulin derivatives have a pH value in the range of 7.2 to 7.4 and mostly buffering agents are used to achieve and maintain the pH within this range.

It has now surprisingly been found that an alternative aqueous pharmaceutical formulation comprising at least one insulin analogue and/or insulin derivative without glycerol shows an excellent chemical and physical stability which qualifies this aqueous pharmaceutical formulation as a medicinal product having a defined shelf life.

SUMMARY OF THE INVENTION

One embodiment of the present invention relates to a pharmaceutical formulation comprising
(a). at least one analogue and/or derivative of insulin; and
(b). Zn(II); and
(c). optionally protamine;
wherein the pharmaceutical formulation is free of glycerol.

In another embodiment, the pharmaceutical formulation according to the present invention is an aqueous pharmaceutical formulation.

In another embodiment, the pharmaceutical formulation according to the present invention comprises at least one analogue and/or derivative of insulin which has or have an isoelectric point (IEP) from 4.0 to 6.0, from 4.5 to 6.0, from 4.5 to 5.5, from 5.0 to 5.5, from 5.0 to 5.2 or 5.1.

In another embodiment, the pharmaceutical formulation according to the present invention has a pH value in the range from 6.0 to 9.0, from 6.5 to 8.5, from 7.0 to 8.0, from 7.0 to 7.8, from 7.1 to 7.6, from 7.2, 7.3, 7.4 or 7.5, or 7.4.

In another embodiment, the pharmaceutical formulation according to the present invention comprises at least one analogue of insulin which is selected from the group consisting of insulin aspart, insulin lispro and/or insulin glulisine. In one embodiment the pharmaceutical formulation according to the present invention comprises an analogue of insulin which is insulin lispro. In one

embodiment the pharmaceutical formulation according to the present invention comprises an analogue of insulin which is insulin aspart. In one embodiment the pharmaceutical formulation according to the present invention comprises an analogue of insulin which is insulin glulisine.

In another embodiment, the pharmaceutical formulation according to the present invention comprises a derivative of insulin which is selected from the group consisting of insulin detemir and/or insulin degludec. In one embodiment the pharmaceutical formulation according to the present invention comprises a derivative of insulin which is insulin detemir. In one embodiment the pharmaceutical formulation according to the present invention comprises a derivative of insulin which is insulin degludec.

In another embodiment, the pharmaceutical formulation according to the present invention comprises at least one analogue and/or derivative of insulin which is present in a concentration from 10 U/mL to 1000 U/mL, from 10 U/mL to 600 U/mL, from 10 U/mL to 300 U/mL, from 50 U/mL to 300 U/mL or 100 U/mL.

In another embodiment, the pharmaceutical formulation according to the present invention comprises an analogue and/or derivative of insulin which is present in a concentration from 60 to 6000 nmol/mL, from 60 nmol/mL to 3600 nmol/mL, from 60 nmol/mL to 1800 nmol/mL, from 300 nmol/mL to 1800 nmol/mL or 600 nmol/mL.

In another embodiment, the pharmaceutical formulation according to the present invention comprises Zn(II) which is present in a concentration from 0.0100 mg/mL to 0.0600 mg/mL, from 0.0150 mg/mL to 0.0500 mg/mL, from 0.0150 mg/mL to 0.0300 mg/mL, from 0.0150 mg/mL to 0.0200 mg/mL, from 0.0190 mg/mL to 0.0200 mg/mL, or 0.0196 mg/mL.

In another embodiment, the pharmaceutical formulation according to the present invention comprises zinc chloride ($ZnCl_2$) or zinc oxide (ZnO) or zinc acetate (anhydrous: $C_4H_6O_4Zn$ or dehydrate $C_4H_6O_4Zn \times 2H_2O$).

In another embodiment, the pharmaceutical formulation according to the present invention comprises Zn(II) which is present in a concentration from 0.0100 mg/100 U to 0.0600 mg/100 U, from 0.0150 mg/100 U to 0.0500 mg/100 U, from 0.0150 mg/100 U to 0.0300 mg/100 U, from 0.0150 mg/100 U to 0.0200 mg/100 U, from 0.0190 mg/100 U to 0.0200 mg/100 U, or 0.0196 mg/100 U.

In another embodiment, the pharmaceutical formulation according to the present invention further comprises sodium chloride which is present in a concentration from 5.0 mg/mL to 15.0 mg/mL, from 5.0 mg/mL to 12.5 mg/mL, from 5.0 mg/mL to 10.0 mg/mL, from 5.0 mg/mL to 9.0 mg/mL, from 5.6 mg/mL to 9.0 mg/mL, from 5.6 mg/mL to 8.5 mg/mL, from 5.6 mg/mL to 8.0 mg/mL, from 5.6 mg/mL to 7.5 mg/mL, from 5.6 mg/mL to 7.0 mg/mL, from 5.6 mg/mL to 6.8 mg/mL, from 5.6 mg/mL to 6.2 mg/mL, 5.6 mg/mL, 6.2 mg/mL, or 6.8 mg/mL.

In another embodiment, the pharmaceutical formulation according to the present invention further contains a buffering agent.

In another embodiment, the pharmaceutical formulation according to the present invention further contains a buffering agent selected from the group comprising 2-amino-2-hydroxymethyl-propane-1,3-diol (TRIS), phosphate, citric acid or citrate salts, acetic acid and salts thereof, glycylglycine and methionine. In one embodiment, the pharmaceutical formulation according to the present invention further contains a buffering agent which is phosphate or Na_2HPO_4 or Na_2HPO_4 x 7 H_2O.

In another embodiment, the pharmaceutical formulation according to the present invention comprises protamine or protamine sulfate which is present in a concentration from 0.10, 0.15, 0.20, 0.25, 0.30, 0.32, 0.35, 0.40, 0.45 or 0.5 mg/mL.

In another embodiment, the pharmaceutical formulation according to the present invention comprises a stabilizing agent, which is in one embodiment a surfactant, a polyoxyethylene derivative of sorbitan monolaurate (e.g. polysorbate 20), a polyethoxylethylene derivate of oleic acid (e.g. polysorbate 80), poloxamer (which is a polyoxyethylene-polyoxypropylene copolymer), or polysorbate 20 or polysorbate 80 or mixtures thereof. In another embodiment, the stabilizing agent, in one embodiment the surfactant, the polyoxyethylene derivative of sorbitan monolaurate (e.g. polysorbate 20), the polyethoxylethylene derivate of oleic acid (e.g. polysorbate 80), poloxamer (which is a polyoxyethylene-polyoxypropylene copolymer), or polysorbate 20 or polysorbate 80 or mixtures thereof are/is present in a concentration from 0.01 to 0.05 mg/mL, in a concentration of 0.010 mg/mL, 0.015 mg/mL, 0.020 mg/mL, 0.025 mg/mL, 0.03 mg/mL, or 0.02 mg/mL.

In a another embodiment, the pharmaceutical formulation according to the present invention comprises more than one analogue and/or derivative of insulin, wherein one analogue and/or

derivative of insulin is a fast acting insulin and one analogue and/or derivative of insulin is a long acting insulin. In another embodiment, the pharmaceutical formulation according to the present invention comprises a fast acting insulin selected from the group comprising insulin aspart, insulin lispro and/or insulin glulisine and and a long acting insulin selected from the group comprising insulin glargin, insulin detemir and/or insulin degludec.

In another embodiment, the pharmaceutical formulation according to the present invention comprises one or more further active pharmaceutical ingredients. In one embodiment, the further active pharmaceutical ingredient is an antidiabetic agent. In another embodiment, the pharmaceutical formulation according to the present invention comprises one or more antidiabetic agents as further active pharmaceutical ingredients selected from the group comprising: GLP-1 receptor agonists, dual GLP-1 receptor/glucagon receptor agonists, human FGF-21, FGF-21 analogues, FGF-21 derivatives, insulins, human insulin, analogues of insulin, and derivatives of insulin. In another embodiment, the pharmaceutical formulation according to the present invention comprises one or more further active pharmaceutical ingredients selected from the group comprising: insulin and insulin derivatives, GLP-1, GLP-1 analogues and GLP-1 receptor agonists, polymer bound GLP-1 and GLP-1 analogues, dual GLP1/GIP agonists, dual GLP1/Glucagon receptor agonists, PYY3-36 or analogues thereof, pancreatic polypeptide or analogues thereof, glucagon receptor agonists or antagonists, GIP receptor agonists or antagonists, ghrelin antagonists or inverse agonists, Xenin and analogues thereof, DDP-IV inhibitors, SGLT2 inhibitors, dual SGLT2 / SGLT1 inhibitors, biguanides thiazolidinediones, dual PPAR agonists, sulfonylureas, meglitinides, alpha-glucosidase inhibitors, amylin and amylin analogues, GPR119 agonists, GPR40 agonists, GPR120 agonists, GPR142 agonists, systemic or low-absorbable TGR5 agonists, Cycloset, inhibitors of 11-beta-HSD, activators of glucokinase, inhibitors of DGAT, inhibitors of protein tyrosinephosphatase 1, inhibitors of glucose-6-phosphatase, inhibitors of fructose-1,6-bisphosphatase, inhibitors of glycogen phosphorylase, inhibitors of phosphoenol pyruvate carboxykinase, inhibitors of glycogen synthase kinase, inhibitors of pyruvate dehydrogenase kinase, alpha2-antagonists, CCR-2 antagonists, modulators of glucose transporter-4, somatostatin receptor 3 agonists, HMG-CoA-reductase inhibitors, fibrates, nicotinic acid and the derivatives thereof, nicotinic acid receptor 1 agonists, PPAR-alpha, gamma or alpha/gamma) agonists or modulators, PPAR-delta agonists, ACAT inhibitors, cholesterol absorption inhibitors, bile acid-binding substances, IBAT inhibitors, MTP inhibitors, modulators of PCSK9, LDL receptor up-regulators by liver selective thyroid hormone receptor ß agonists, HDL-raising compounds, lipid metabolism modulators, PLA2 inhibitors , ApoA-I enhancers, cholesterol

synthesis inhibitors, lipid metabolism modulators, omega-3 fatty acids and derivatives thereof, active substances for the treatment of obesity, such as sibutramine, tesofensine, orlistat, CB-1receptor antagonists, MCH-1 antagonists, MC4 receptor agonists and partial agonists, NPY5 or NPY2 antagonists, NPY4 agonists, beta-3-agonists, leptin or leptin mimetics, agonists of the 5HT2c receptor, or the combinations of bupropione/naltrexone (CONTRAVE), bupropione/zonisamide (EMPATIC), bupropione/phentermine or pramlintide/metreleptin, QNEXA (Phentermine+ topiramate), lipase inhibitors, angiogenesis inhibitors, H3 antagonists, AgRP inhibitors, triple monoamine uptake inhibitors (norepinephrine and acetylcholine), MetAP2 inhibitors, nasal formulation of the calcium channel blocker diltiazem, antisense oligonucleotides against production of fibroblast growth factor receptor 4, prohibitin targeting peptide-1, drugs for influencing high blood pressure, chronic heart failure or atherosclerosis, such as angiotensin II receptor antagonists, ACE inhibitors, ECE inhibitors, diuretics, beta-blockers, calcium antagonists, centrally acting hypertensives, antagonists of the alpha-2-adrenergic receptor, inhibitors of neutral endopeptidase, thrombocyte aggregation inhibitors.

In another embodiment, the pharmaceutical formulation according to the present invention comprises more than one analogue and/or derivative of insulin, wherein one analogue and/or derivative of insulin is a fast acting insulin and one analogue and/or derivative of insulin is a long acting insulin. In one embodiment, the fast acting insulin is selected from the group comprising insulin aspart, insulin lispro and/or insulin glulisine and wherein the long acting insulin is selected from the group comprising insulin detemir and/or insulin degludec.

In another embodiment, the pharmaceutical formulation according to the present invention consists of (a). 3.5 mg/mL insulin aspart; and (b). 1.72 mg/mL metacresol; and (c). 1.50 mg/mL phenol; and (d). 0.0196 mg/mL Zn(II); and (e). 6.2 mg/mL sodium chloride; and (f). 1.88 mg/mL Na_2HPO_4 x 7 H_2O; and (g). sodium hydroxide and/or hydrochloric acid to adjust the pH to 7.4; and (i). water.

In another embodiment, the pharmaceutical formulation according to the present invention consists of (a). 3.5 mg/mL insulin aspart; and (b). 1.72 mg/mL metacresol; and (c). 1.50 mg/mL phenol; and (d). 0.0196 mg/mL Zn(II); and (e). 6.2 mg/mL sodium chloride; and (f). 1.88 mg/mL Na_2HPO_4 x 7 H_2O; and (g). from 0.1 mg/mL to 0.5 mg/mL protamine; (h). sodium hydroxide and/or hydrochloric acid to adjust the pH to a pH in the range from 7.1 to 7.6 and water.

The present invention also provides a pharmaceutical formulation according to the present invention for use in the treatment of diabetes mellitus, hyperglycemia and/or for use in lowering blood glucose levels.

The present invention also provides a process for preparing the pharmaceutical formulation according to the present invention, wherein the components are mixed together in the form of a solution or suspension, the desired pH is adjusted and the mixture is made up to the final volume with water.

The present invention also relates to a kit or combination comprising separate packages of the pharmaceutical formulation according to the present invention and a medical device. In one embodiment, the medical device is selected from the group comprising: syringe, insulin injection system, insulin infusion system, insulin pump, insulin pen injection device.

The present invention also relates to a kit or combination comprising separate packages of the pharmaceutical formulation according to the present invention, of at least one further active pharmaceutical ingredient and optionally of a medical device. In one embodiment, the medical device is selected from the group comprising: syringe, insulin injection system, insulin infusion system, insulin pump, insulin pen injection device.

The present invention also relates to a kit or combination comprising separate packages of the pharmaceutical formulation according to the present invention, of at least one further active pharmaceutical ingredient and optionally of a medical device, wherein the further active pharmaceutical ingredient is an antidiabetic agent.

The present invention also relates to a kit or combination comprising separate packages of the pharmaceutical formulation according to the present invention, of at least one further active pharmaceutical ingredient and optionally of a medical device, wherein the further active pharmaceutical ingredient is an antidiabetic agent selected from the group comprising: GLP-1 receptor agonists, dual GLP-1 receptor/glucagon receptor agonists, human FGF-21, FGF-21 analogues, FGF-21 derivatives, insulins, human insulin, analogues of insulin, and derivatives of insulin. In another embodiment, the pharmaceutical formulation according to the present invention comprises one or more further active pharmaceutical ingredients selected from the group comprising: insulin and insulin derivatives, GLP-1, GLP-1 analogues and GLP-1 receptor agonists,

polymer bound GLP-1 and GLP-1 analogues, dual GLP1/GIP agonists, dual GLP1/Glucagon receptor agonists, PYY3-36 or analogues thereof, pancreatic polypeptide or analogues thereof, glucagon receptor agonists or antagonists, GIP receptor agonists or antagonists, ghrelin antagonists or inverse agonists, Xenin and analogues thereof, DDP-IV inhibitors, SGLT2 inhibitors, dual SGLT2 / SGLT1 inhibitors, biguanides thiazolidinediones, dual PPAR agonists, sulfonylureas, meglitinides, alpha-glucosidase inhibitors, amylin and amylin analogues, GPR119 agonists, GPR40 agonists, GPR120 agonists, GPR142 agonists, systemic or low-absorbable TGR5 agonists, Cycloset, inhibitors of 11-beta-HSD, activators of glucokinase, inhibitors of DGAT, inhibitors of protein tyrosinephosphatase 1, inhibitors of glucose-6-phosphatase, inhibitors of fructose-1,6-bisphosphatase, inhibitors of glycogen phosphorylase, inhibitors of phosphoenol pyruvate carboxykinase, inhibitors of glycogen synthase kinase, inhibitors of pyruvate dehydrogenase kinase, alpha2-antagonists, CCR-2 antagonists, modulators of glucose transporter-4, somatostatin receptor 3 agonists, HMG-CoA-reductase inhibitors, fibrates, nicotinic acid and the derivatives thereof, nicotinic acid receptor 1 agonists, PPAR-alpha, gamma or alpha/gamma) agonists or modulators, PPAR-delta agonists, ACAT inhibitors, cholesterol absorption inhibitors, bile acid-binding substances, IBAT inhibitors, MTP inhibitors, modulators of PCSK9, LDL receptor up-regulators by liver selective thyroid hormone receptor ß agonists, HDL-raising compounds, lipid metabolism modulators, PLA2 inhibitors , ApoA-I enhancers, cholesterol synthesis inhibitors, lipid metabolism modulators, omega-3 fatty acids and derivatives thereof, active substances for the treatment of obesity, such as sibutramine, tesofensine, orlistat, CB-1receptor antagonists, MCH-1 antagonists, MC4 receptor agonists and partial agonists, NPY5 or NPY2 antagonists, NPY4 agonists, beta-3-agonists, leptin or leptin mimetics, agonists of the 5HT2c receptor, or the combinations of bupropione/naltrexone (CONTRAVE), bupropione/zonisamide (EMPATIC), bupropione/phentermine or pramlintide/metreleptin, QNEXA (Phentermine+ topiramate), lipase inhibitors, angiogenesis inhibitors, H3 antagonists, AgRP inhibitors, triple monoamine uptake inhibitors (norepinephrine and acetylcholine), MetAP2 inhibitors, nasal formulation of the calcium channel blocker diltiazem, antisense oligonucleotides against production of fibroblast growth factor receptor 4, prohibitin targeting peptide-1, drugs for influencing high blood pressure, chronic heart failure or atherosclerosis, such as angiotensin II receptor antagonists, ACE inhibitors, ECE inhibitors, diuretics, beta-blockers, calcium antagonists, centrally acting hypertensives, antagonists of the alpha-2-adrenergic receptor, inhibitors of neutral endopeptidase, thrombocyte aggregation inhibitors.

The present invention also relates to a kit or combination comprising separate packages of the pharmaceutical formulation according to the present invention, of at least one further active

pharmaceutical ingredient and optionally of a medical device, wherein the kit comprises more than one analogue and/or derivative of insulin, wherein one analogue and/or derivative of insulin is a fast acting insulin and one analogue and/or derivative of insulin is a long acting insulin. In one embodiment, the fast acting insulin is selected from the group comprising insulin aspart, insulin lispro and/or insulin glulisine and wherein the long acting insulin is selected from the group comprising insulin glargin, insulin detemir and/or insulin degludec.

The present invention also relates to a kit or combination comprising separate packages of the pharmaceutical formulation according to the present invention, of at least one further active pharmaceutical ingredient and optionally of a medical device for use in the treatment of diabetes mellitus, hyperglycemia and/or for use in lowering blood glucose levels.

In another embodiment, the present invention also relates to a kit or combination comprising separate packages of the pharmaceutical formulation according to the present invention, of at least one further active pharmaceutical ingredient and optionally of a medical device, wherein the pharmaceutical formulation according to the present invention and the further active pharmaceutical ingredient, in one embodiment an antidiabetic agent, are administered continuously, separately, sequentially and/or stepwise.

The present invention also relates to the use of a medical device for administering the pharmaceutical formulation to an animal and/or human. In another embodiment, the medical device is selected from the group comprising: syringe, insulin injection system, insulin infusion system, insulin pump, insulin pen injection device

DETAILED DESCRIPTION

As used herein, the singular forms “a”, “an”, and “the” include plural reference unless the context clearly dictates otherwise. Thus, for example, reference to a fill material containing “a carrier” includes one or more carriers, reference to “an additive” includes reference to one or more of such additives.

As used herein, the term “active pharmaceutical ingredient” (API) includes any pharmaceutically active chemical or biological compound and any pharmaceutically acceptable salt thereof and any mixture thereof, that provides some pharmacologic effect and is used for treating or preventing a condition. Exemplary pharmaceutically acceptable salts include hydrochloric, sulfuric, nitric, phosphoric, hydrobromic, maleric, malic, ascorbic, citric, tartaric, pamoic, lauric, stearic, palmitic,

oleic, myristic, lauryl sulfuric, naphthalinesulfonic, linoleic, linolenic acid, and the like. As used herein, the terms “active pharmaceutical ingredient”, “drug”, “active agent”, “active ingredient”, “active substance” and “drug” are meant to be synonyms, i.e., have identical meaning.

In one embodiment the active pharmaceutical ingredient is an antidiabetic agent. Examples of antidiabetic are found in the Rote Liste 2012, chapter 12. Examples of antidiabetic agents include but are not limited to (a) insulin, insulin analogues and insulin derivatives, (b) glucagon-like-peptide 1 (GLP-1) and its analogues and receptor agonists, (c) dual GLP-1/GIP agonists, and (d) dual GLP-1/glucagon receptor agonists, as described in detail next.

(a). Insulin, insulin analogues and insulin derivatives

Examples of insulin, insulin analogues, and insulin derivatives include but are not limited to: insulin glargine (Lantus®), insulin glulisine (Apidra®), insulin detemir (Levemir®), insulin lispro (Humalog® / Liprolog®), insulin degludec (Tresiba®), insulin aspart (NovoLog® / NovoRapid®), basal insulin and analogues (e.g. LY2605541, LY2963016), PEGylated insulin lispro, Humulin®, Linjeta®, SuliXen®, NN1045, insulin plus Symlin®, fast-acting and short-acting insulins (e.g. Linjeta®, PH20 insulin, NN1218, HinsBet®), oral, inhalable, transdermal and sublingual insulins (e.g. Exubera®, Nasulin®, Afrezza®, insulin tregopil, TPM -02/Insulin, Capsulin®, Oral-lyn®, Cobalamin® oral insulin, ORMD-0801, NN1953, VIAtab®). Additionally included are also those insulin derivatives which are bonded to albumin or another protein by a bifunctional linker.

(b). Glucagon-like-peptide 1 (GLP-1), GLP-1 analogues and GLP-1 receptor agonists

Examples of GLP-1, GLP-1 analogues and GLP-1 receptor agonists include but are not limited to lixisenatide (AVE0010 / ZP10 / Lyxumia®), exenatide / exendin-4 (Byetta® / Bydureon® / ITCA 650, liraglutide / Victoza®), semaglutide, taspoglutide, albiglutide, dulaglutide, rExendin-4, CJC-1134-PC, PB-1023, TTP-054, HM-11260C, CM-3, GLP-1 Eligen, ORMD-0901, NN9924, Nodexen, Viador-GLP-1, CVX-096, ZYOG-1, ZYD-1, MAR-701, ZP-2929, ZP-3022, CAM-2036, DA-15864, ARI-2651, ARI-2255, exenatide-XTEN and glucagon-XTEN, AMX-8089+VRS-859 and polymer bound GLP-1 and GLP-1 analogues.

(c). Dual GLP-1/ glucose-dependent insulinotropic peptides (GIP) agonists

Examples of dual GLP-1/GIP agonists include but are not limited to MAR701, MAR-709, BHM081 / BHM089 / BHM098).

(d). Dual GLP-1/glucagon receptor agonists

Examples of dual GLP-1/glucagon receptor agonists include but are not limited to OAP-189 (PF-05212389, TKS-1225), TT-401/402, ZP2929, LAPS-HMOXM25, MOD-6030).

Other suitable active pharmaceutical ingredients which may be included in the pharmaceutical formulations of the invention include but are not limited to the following:

Further gastrointestinal peptides such as peptide YY 3-36 (PYY3-36) or analogues thereof and pancreatic polypeptide (PP) or analogues thereof.

Glucagon receptor agonists or antagonists, GIP receptor agonists or antagonists, ghrelin antagonists or inverse agonists and xenin and analogues thereof.

Dipeptidyl peptidase-IV (DPP-4) inhibitors, for example: alogliptin / Nesina®, linagliptin / BI-1356 / Ondero® / Trajenta® / Tradjenta® / Trayenta®, saxagliptin / Onglyza®, sitagliptin / Januvia® / Xelevia® / Tesavel®, sitagliptin + metformin / Janumet® / Velmetia®, aildagliptin, anagliptin, aemigliptin, tenegliptin, melogliptin, trelagliptin, DA-1229, MK-3102, KM-223, KRP-104 and Ari-2243.

Sodium-dependent glucose transporter 2 (SGLT2) inhibitors, for example: canagliflozin, dapagliflozin, remogliflozin, sergliflozin, empagliflozin, ipragliflozin, tofogliflozin (RO-4998452), luseogliflozin, LX-4211, ertugliflozin (PF-04971729), EGT-0001442 and DSP-3235.

Dual SGLT2 / SGLT1 inhibitors.

Biguanides (e.g. metformin, buformin, phenformin), thiazolidinediones (e.g. pioglitazone, rivoglitazone, rosiglitazone, troglitazone), dual PPAR agonists (e.g. aleglitazar, muraglitazar, tesaglitazar), sulfonylureas (e.g. tolbutamide, glibenclamide, glimepiride / Amaryl®, glipizide), meglitinides (e.g. nateglinide, repaglinide, mitiglinide), alpha-glucosidase inhibitors (e.g. acarbose, miglitol, voglibose), amylin and amylin analogues (e.g. pramlintide / Symlin®).

G-protein coupled receptor 119 (GPR119) agonists (e.g. GSK-1292263, PSN-821, MBX-2982, APD-597, ARRY-981).

GPR40 agonists (e.g. TAK-875, TUG-424, P-1736, JTT-851, GW9508).

GPR120 agonists and GPR142 agonists.

Systemic or low-absorbable TGR5 (GPBAR1 = G-protein-coupled bile acid receptor 1) agonists (e.g. INT-777, XL-475, SB756050).

Bromocriptine / Cycloset®, inhibitors of 11-beta-hydroxysteroid dehydrogenase (11-beta-HSD) (e.g. LY2523199, BMS770767, RG-4929, BMS816336, AZD-8329, HSD-016, BI-135585), activators of glucokinase (e.g. PF-04991532, TTP-399, GK1-399, ARRY-403 (AMG-151), TAK-329, ZYGK1), inhibitors of diacylglycerol O-acyltransferase (DGAT) (e.g. pradigastat (LCQ-908), LCQ-908), inhibitors of protein tyrosinephosphatase 1 (e.g. trodusquemine), inhibitors of glucose-6-

phosphatase, inhibitors of fructose-1,6-bisphosphatase, inhibitors of glycogen phosphorylase, inhibitors of phosphoenol pyruvate carboxykinase, inhibitors of glycogen synthase kinase, inhibitors of pyruvate dehydrogenase kinase, alpha2 adrenergic receptor antagonists, C-C chemokine receptor type 2 (CCR-2) antagonists, modulators of glucose transporter-4 and somatostatin receptor 3 agonists (e.g. MK-4256).

One or more lipid lowering agents are also suitable as active pharmaceutical ingredients, such as for example: 3-hydroxy-3-methylglutaryl-coenzym-A-reductase (HMG-CoA-reductase) inhibitors (e.g. simvastatin, atorvastatin, rosuvastatin), fibrates (e.g. bezafibrate, fenofibrate), nicotinic acid and derivatives thereof (e.g. niacin, including slow release formulations of niacin), nicotinic acid receptor 1 agonists (e.g. GSK-256073), peroxisome proliferator-activated receptors (PPAR-)(alpha, gamma or alpha/gamma) agonists or modulators (e.g. aleglitazar), PPAR-delta agonists, acetyl-CoA−acetyltransferase (ACAT) inhibitors (e.g. avasimibe), cholesterol absorption inhibitors (e.g. ezetimibe), bile acid-binding substances (e.g. cholestyramine, colesevelam), ileal bile acid transport inhibitors (IBAT) (e.g. GSK-2330672), microsomal triglyceride transfer protein (MTP) inhibitors (e.g. lomitapide (AEGR-733), SLx-4090, granotapide), modulators of proprotein convertase subtilisin/kexin type 9 (PCSK9) (e.g. REGN727/SAR236553, AMG-145, LGT-209, PF-04950615, MPSK3169A, LY3015014, ALD-306, ALN-PCS, BMS-962476, SPC5001, ISIS-394814, 1B20, LGT-210, 1D05, BMS-PCSK9Rx-2, SX-PCK9, RG7652), LDL receptor up-regulators, for example liver selective thyroid hormone receptor beta agonists (e.g. eprotirome (KB-2115), MB07811, sobetirome (QRX-431), VIA-3196, ZYT1), HDL-raising compounds such as: CETP inhibitors (e.g. torcetrapib, anacetrapib (MK0859), dalcetrapib, evacetrapib, JTT-302, DRL-17822, TA-8995, R-1658, LY-2484595) or ABC1 regulators, lipid metabolism modulators (e.g. BMS-823778, TAP-301, DRL-21994, DRL-21995), phospholipase A2 (PLA2) inhibitors (e.g. darapladib / Tyrisa®, varespladib, rilapladib), ApoA-I enhancers (e.g. RVX-208 , CER-001 , MDCO-216, CSL-112, VRX-HDL, VRX-1243, VIRxSYS), cholesterol synthesis inhibitors (e.g. ETC-1002) and lipid metabolism modulators (e.g. BMS-823778, TAP-301, DRL-21994, DRL-21995) and omega-3 fatty acids and derivatives thereof (e.g. icosapent ethyl (AMR101), Epanova®, AKR-063, NKPL-66).

Other suitable active pharmaceutical ingredients which may be included in the pharmaceutical formulations include one or more active substances for the treatment of obesity, including but not limited to:

Sibutramine, tesofensine, orlistat, cannabinoid receptor 1 (CB1) antagonists (e.g. TM-38837), melanin-concentrating hormone (MCH-1) antagonists (e.g. BMS-830216, ALB-127158(a)), MC4 receptor agonists and partial agonists (e.g. AZD-2820, RM-493), neuropeptide Y5 (NPY5) or NPY2 antagonists (e.g. velneperit, S-234462), NPY4 agonists (e.g. PP-1420), beta-3-

adrenergic receptor agonists, leptin or leptin mimetics, agonists of the 5-hydroxytryptamine 2c (5HT2c) receptor (e.g. lorcaserin), or the combinations of bupropione/naltrexone (Contrave®), bupropione/zonisamide (Empatic®), bupropione/phentermine or pramlintide/metreleptin, phentermine/topiramate (Qsymia®), lipase inhibitors (e.g. cetilistat / Cametor®), angiogenesis inhibitors (e.g. ALS-L1023), histamine H3 antagonists (e.g. HPP-404), AgRP (agouti related protein) inhibitors (e.g. TTP-435), triple monoamine uptake inhibitors (dopamine, norepinephrine and serotonin reuptake) (e.g. tesofensine), methionine aminopeptidase 2 (MetAP2) inhibitors (e.g. beloranib), nasal formulations of the calcium channel blocker diltiazem (e.g. CP-404) and antisense oligonucleotides against production of fibroblast growth factor receptor 4 (FGFR4) (e.g. ISIS-FGFR4Rx) or prohibitin targeting peptide-1 (e.g. Adipotide®).

Further suitable active pharmaceutical ingredients which may be included in the pharmaceutical formulations include but are not limited to:
Angiotensin II receptor antagonists (e.g. telmisartan, candesartan, valsartan, losartan, eprosartan, irbesartan, olmesartan, tasosartan, azilsartan), angiotensin converting enzyme (ACE) inhibitors, endothelin converting enzyme (ECE) inhibitors, diuretics, beta-blockers, calcium antagonists, centrally acting hypertensives, antagonists of the alpha-2-adrenergic receptor, inhibitors of neutral endopeptidase, thrombocyte aggregation inhibitors and others or combinations thereof are suitable.

As used herein, the terms „analogue of insulin" and "insulin analogue" refers to a polypeptide which has a molecular structure which formally can be derived from the structure of a naturally occurring insulin, for example that of human insulin, by deleting and/or exchanging at least one amino acid residue occurring in the naturally occurring insulin and/or adding at least one amino acid residue. The added and/or exchanged amino acid residue can either be codable amino acid residues or other naturally occurring residues or purely synthetic amino acid residues. Examples of analogues of insulin include, but are not limited to, the following: (i). ´Insulin aspart´ is created through recombinant DNA technology so that the amino acid B28 in human insulin (i.e. the amino acid no. 28 in the B chain of human insulin), which is proline, is replaced by aspartic acid; (ii). ´Insulin lispro´ is created through recombinant DNA technology so that the penultimate lysine and proline residues on the C-terminal end of the B-chain of human insulin are reversed (human insulin: $Pro^{B28}Lys^{B29}$; insulin lispro: $Lys^{B28}Pro^{B29}$); (iii). ´Insulin glulisine´ differs from human insulin in that the amino acid asparagine at position B3 is replaced by lysine and the lysine in position B29 is replaced by glutamic acid; (iv). "Insulin glargine" differs from human insulin in that the

asparagine at position A21 is replaced by glycine and the B chain is extended at the carboxy terminal by two arginines.

As used herein, the term “aqueous” refers to a solution in which the solvent is water and/or to a suspension in which the external phase is water and/or to an emulsion in which the dispersed or continuous phase is water.

As used herein, the term “buffering agent” refers to a weak acid or base used to maintain the acidity (pH) of a solution, a suspension and/or an emulsion near a chosen value after the addition of another acid or base. The function of a buffering agent is to prevent a rapid change in the pH value when acids or bases are added to the solution. In an aqueous solution, suspension and/or emulsion, a buffering agent is present in a mixture of a weak acid and its conjugate base or a in a mixture of a weak base and its conjugated acid. Examples of buffering agents include, but are not limited to, the following: sodium bicarbonate; acetic acid or acetate salts (e.g. sodium acetate, zinc acetate); boric acid or boric salts; N-cyclohexyl-2-aminoethanesulfonic acid (CHES) or salts thereof; 3-[[1,3-dihydroxy-2-(hydroxymethyl)propan-2-yl]amino]propane-1-sulfonic acid (TAPS) or salts thereof; 2-(N-morpholino)ethanesulfonic acid (MES) and salts therof; piperazine-N,N′-bis(2-ethanesulfonic acid (PIPES) and salts therof; N-(2-acetamido)-2-aminoethane-sulfonic acid (ACES) and salts therof; cholamine chloride; BES; 2-[[1,3-dihydroxy-2-(hydroxymethyl)-propan-2-yl]amino]ethanesulfonic acid (TES) and salts therof; 2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid (HEPES) and salts therof; acetamidoglycine; N-(2-hydroxy-1,1-bis(hydroxyl-methyl)ethyl)glycine (tricine); glycinamide; 2-(bis(2-hydroxyethyl)amino)acetic acid (bicine) and salts therof; propionate salts; 3-[[1,3-dihydroxy-2-(hydroxymethyl)propan-2-yl]-amino]-2-hydroxy-propane-1-sulfonic acid (TAPSO) and salts therof; 3-morpholinopropane-1-sulfonic acid (MOPS) and salts therof; saline-sodium citrate (SSC) buffer; 2-amino-2-hydroxymethyl-propane-1,3-diol (synonyms: TRIS, trisamine, THAM, tromethamine, trometamol, tromethane); citric acid or citrate salts (e.g. sodium citrate); trisodium phosphate, disodium hydrogen phosphate, sodium dihydrogen phosphate, tripotassium phosphate, dipotassium phosphate, monopotassium phosphate and/or any other buffering agent containing phosphate.

Amino acids (having free basic or acidic functional groups, e.g. methionin, arginine) or peptides (having free basic or acidic functional groups) may also be used as buffering agent. As used herein, the term “buffering agent” also comprises amino acids, peptides and proteins. As insulin analogues and/or insulin derivatives and/or protamine are peptides or derivatives of peptides (i.e. both contain amino acids having free basic or acidic functional groups), they may also have a certain buffering capacity, i.e. are also to be considered as buffering agent.

As used herein, the term “fast acting insulin” or “short acting insulin” refers to insulin analogues and/or insulin derivatives, wherein the insulin-mediated effect begins within 5 to 15 minutes and continues to be active for 3 to 4 hours. Examples of fast acting insulins include, but are not limited to, the following: (i). insulin aspart; (ii). insulin lispro and (iii). insulin glulisine.

As used throughout the description and the claims of this specification, the word “comprise” and variations of the word, such as “comprising” and “comprises” is not intended to exclude other additives, components, integers or steps.

As used herein, the terms „derivative of insulin” and “insulin derivative” refers to a polypeptide which has a molecular structure which formally can be derived from the structure of a naturally occurring insulin, for example that of human insulin, in which one or more organic substituent (e.g. a fatty acid) is bound to one or more of the amino acids. Optionally, one or more amino acids occurring in the naturally occurring insulin may have been deleted and/or replaced by other amino acids, including non-codeable amino acids, or amino acids, including non-codeable, have been added to the naturally occurring insulin. Examples of derivatives of insulin include, but are not limited to, the following: (i). ´Insulin detemir´ which differs from human insulin in that the C-terminal threonine in position B30 is removed and a fatty acid residue (myristic acid) is attached to the epsilon-amino function of the lysine in position B29. (ii). ´Insulin degludec´ which differs from human insulin in that the last amino acid is deleted from the B-chain and by the addition of a glutamyl link from Lys^{B29} to a hexadecandioic acid.

As used herein, the term “FGF-21” means “fibroblast growth factor 21”. FGF-21 compounds may be human FGF-21, an analogue of FGF-21 (referred to “FGF-21 analogue”) or a derivative of FGF-21 (referred to “FGF-21 derivative”).

As used herein, the term "formulation" refers to a product comprising specified ingredients in predetermined amounts or proportions, as well as any product that results, directly or indirectly, from combining specified ingredients in specified amounts. In relation to pharmaceutical formulations, this term encompasses a product comprising one or more active ingredients, and an optional carrier comprising inert ingredients, as well as any product that results, directly or indirectly, from combination, complexation or aggregation of any two or more of the ingredients, or from dissociation of one or more of the ingredients, or from other types of reactions or interactions of one or more of the ingredients. In general, pharmaceutical formulations are prepared by

uniformly bringing the active pharmaceutical ingredient (i.e. the analogue and/or derivative of insulin) into association with a liquid carrier or a finely divided solid carrier or both, and then, if necessary, shaping the product into the desired formulation. The pharmaceutical formulation includes enough of the active pharmaceutical ingredient to produce the desired effect upon the progress or condition of diseases. As used herein, the term "formulation" may refer to a solution as well as to a suspension or to an emulsion. As used herein, the terms "formulation" and "composition" are meant to be synonyms, i.e., have identical meaning. The pharmaceutical compositions are made following conventional techniques of pharmaceutical technology involving mixing, filling and dissolving the ingredients, as appropriate, to give the desired oral, parenteral, rectal, transdermal, or topical products.

As used herein, the term "GLP-1 receptor agonist" refers to compounds which have an agonistic activity at the glucagon-like peptide-1 receptor. Examples of GLP-1 receptor agonists include, but are not limited to, the following: exenatide / exendin-4, liraglutide, lixisenatide, dulaglutide, albiglutide, semaglutide, taspoglutide, rExendin-4, CJC-1134-PC, PB-1023, TTP-054, HM-11260C, CM-3, GLP-1 Eligen, ORMD-0901, NN9924, Nodexen, Viador-GLP-1, CVX-096, ZYOG-1, ZYD-1, MAR-701, ZP-2929, ZP-3022, CAM-2036, DA-15864, ARI-2651, ARI-2255, exenatide-XTEN and glucagon-XTEN, AMX-8089+VRS-859 and polymer bound GLP-1 and GLP-1 analogues.

As used herein, the term "dual GLP-1 receptor/glucagon receptor agonist" refers to compounds which have agonistic activity at both the GLP-1 receptor and the glucacon receptor. Examples of dual GLP-1 receptor/glucagon receptor agonist include, but are not limited to, the following: oxyntomodulin, MAR701, MAR-709, and BHM081 / BHM089 / BHM098.

As used herein, the term "human insulin" refers to the human hormone whose structure and properties are well-known. Human insulin has two polypeptide chains (chains A and B) that are connected by disulphide bridges between cysteine residues, namely the A-chain and the B-chain. The A-chain is a 21 amino acid peptide and the B-chain is a 30 amino acid peptide, the two chains being connected by three disulphide bridges: one between the cysteins in position 6 and 11 of the A-chain; the second between the cysteine in position 7 of the A-chain and the cysteine in position 7 of the B-chain; and the third between the cysteine in position 20 of the A-chain and the cysteine in position 19 of the B-chain.

As used herein, the term "including" is used to mean "including but not limited to". "Including" and "including but not limited to" are used interchangeably.

As used herein, the term “isoelectric point” (pI, IEP) refers to the pH value at which a particular molecule carries no net electrical charge. The isoelectric point can be determined by using isoelectric focusing, which is a technique for separating different molecules by differences in their isoelectric point and which is well known in the art. It can also be calculated (see e.g. Levene and Simms, ´Calculation of isoelectric point´ J. Biol. Chem., 1923, pp. 801-813).

As used herein, the term “kit” refers to a product (e.g. medicament, kit-of-parts) comprising one package or one or moreseparate packages of:
(i). A pharmaceutical formulation containing an active pharmaceutical ingredient and at least one further active pharmaceutical ingredient and optionally a medical device. The at least one further active pharmaceutical ingredient may be present in said pharmaceutical formulation, i.e. the kit may comprise one or more packages, wherein each package comprises one pharmaceutical formulation which comprises two or more active pharmaceutical ingredients. The further active pharmaceutical ingredient may also be present in a further pharmaceutical formulation, i.e. the kit may comprise separate packages of two or more pharmaceutical formulations, wherein each pharmaceutical formulation contain one active pharmaceutical ingredient.
Or
(ii). A pharmaceutical formulation containing an active pharmaceutical ingredient and medical device.

A kit may comprise one package only or may comprise one or more separate packages
For example, the kit may be a product (e.g. medicament) containing two or more vials each containing a defined pharmaceutical formulation, wherein each pharmaceutical formulation contains at least one active pharmaceutical ingredient. For example, the kit may comprise (i.) a vial containing a defined pharmaceutical formulation and (ii). further a tablet, capsule, powder or any other oral dosage form which contains at least one further active pharmaceutical ingredient. The kit may further comprise a package leaflet with instructions for how to administer the pharmaceutical formulation and the at least one further active pharmaceutical ingredient.

As used herein, the term “medical device” means any instrument, apparatus, implant, in vitro reagent or similar or related article that is used to diagnose, prevent, or treat a disease of other condition, and does not achieve its purpose through pharmacological action within or on the body. As used herein, a medical device may be a syringe, an insulin injection system, an insulin infusion

system, an insulin pump or an insulin pen injection device. As used herein, a medical device may be mechanically or electromechanically driven.

As used herein, unless specifically indicated otherwise, the conjunction “or” is used in the inclusive sense of “and/or” and not the exclusive sense of “either/or”.

As used herein, the term “pH” and “pH value” refer to the decimal logarithm of the reciprocal of the hydrogen ion activity in a solution.

As used herein, the term “pharmaceutical” refers to the intended use in the medical diagnosis, cure, treatment and/or prevention of diseases.

As used herein, the term “pharmaceutically acceptable” refers to physiologically well tolerated by a mammal or a human.

As used herein, the term “protamine” refers to a mixture of strongly basic peptides. It was originally isolated from the sperm of salmon and other species of fish but is now produced primarily recombinant through biotechnology. It contains more than two-thirds of L-arginine. As protamine contains amino acids having free basic side chains, it has a certain buffering capacity and is therefore considered to be a buffering agent. Protamine may be used as protamine sulfate and protamine hydrochloride.

Concentrations, amounts, solubilities, particle size, wavelength, pH values, weight mass, molecular weight, percent and other numerical date may be expressed or presented herein in a range format. It is to be understood that such a range format is used merely for convenience and brevity and thus should be interpreted flexibly to include not only the numerical values explicitly recited as the limits of the range, but also to include all the individual numerical values or sub-ranges encompassed within that range as if each numerical value and sub-range is explicitly recited.

As used herein, the term “long acting insulin” refers to insulin analogues and/or insulin derivatives, wherein the insulin-mediated effect begins within 0.5 to 2 hours and continues to be active, for about or more than 24 hours. Examples of fast acting insulins include, but are not limited to, the following: (i). insulin glargin; (ii). insuline detemir and (iii). insulin degludec.

As used herein, the term “stability” refers to the chemical and/or physical stability of active pharmaceutical ingredients, in particular of insulin analogues and/or derivatives. The purpose of stability testing is to provide evidence on how the quality of an active pharmaceutical ingredient or dosage form varies with time under the influence of a variety of environmental factors such as temperature, humidity, and light, and to establish a shelf life for the active pharmaceutical ingredient or dosage form and recommended storage conditions. Stability studies can include testing of those attributes of the active pharmaceutical ingredient that are susceptible to change during storage and are likely to influence quality, safety, and/or efficacy. The testing can cover, as appropriate, the physical, chemical, biological, and microbiological attributes, preservative content (e.g., antioxidant, antimicrobial preservative), and functionality tests (e.g. for a dose delivery system). Analytical procedures can be fully validated and stability indicating. In general, significant changes for an active pharmaceutical ingredient and/or dosage form with regard to stability are defined as:

- a 5% change in assay from its initial value; or failure to meet the acceptance criteria for potency when using biological or immunological procedures;
- any degradation products exceeding its acceptance criterion;
- failure to meet the acceptance criteria for appearance, physical attributes, and functionality test (e.g., color, phase, separation, resuspendibility, caking, hardness, dose delivery per actuation); however, some changes in physical attributes (e.g. softening of suppositories, melting of creams) may be expected under accelerated conditions;

and, as appropriate for the dosage form:

- failure to meet the acceptance criterion for pH; or
- failure to meet the acceptance criteria for dissolution for 12 dosage units.

The significant changes may also be evaluated against established acceptance criteria prior to starting the evaluation of the stability.

Acceptance criteria can be derived from the monographs (e.g. monographs for the European Pharmacopeia, of the United States Pharmacopeia, of the British Pharmacopeia, or others), and from the analytical batches of the active pharmaceutical ingredient and medicinal product used in the preclinical and clinical studies. Acceptable limits should be proposed and justified, taking into account the levels observed in material used in preclinical and clinical studies. Product characteristics may be visual appearance, purity, color and clarity for solutions/suspensions, visible particulates in solutions, and pH. As a non-limiting example, suitable acceptance criteria for insulin aspart formulations are shown below:

Test item	Acceptance criteria for clinical trials
Appearance of solution (visual)	
- Clarity and degree of opalescence	Monitoring
- Degree of coloration	Monitoring
Assay insulin aspart units (HPLC)	90.0 insulin aspart units/mL to 110.0 insulin aspart units/mL
Related impurities (HPLC)	
- B28isoAsp insulin aspart	equal or below to 2.5 %
- Total of A21Asp insulin aspart, B3Asp insulin aspart and B3isoAsp insulin aspart	equal or below to 5.0 %
- Any other unspecified, unidentified impurity	equal or below to 2.0 %
- Total of other impurities	equal or below to 3.5 %
High molecular weight proteins (HPSEC)	equal or below to 1.5 %
pH	7.0 to 7.8
Particulate matter (visible particles)	Practically free of visible particles
Particulate matter (subvisible particles)	Number of particles per container: equal or larger to 10 µm: equal or below to 6000 equal or larger to 25 µm: equal or below to 600
Assay m-cresol	1.55 to 1.89 [mg/mL]
Assay phenol	1.35 to 1.65 [mg/mL]
Zinc (Zn(II)) (AAS)	below 40 µg per 100 units insulin aspart

The acceptance criteria shown above are based on monographed acceptance limits (e.g. British Pharmacopoeia, Volume III, 2012 or Pharmacopoeial Forum, Volume 36(6), Nov-Dec 2010) and/or are derived from extensive experience in the development of insulin formulations.

As used herein, the term "treatment" refers to any treatment of a mammalian, for example human condition or disease, and includes: (1) inhibiting the disease or condition, i.e., arresting its development, (2) relieving the disease or condition, i.e., causing the condition to regress, or (3) stopping the symptoms of the disease.

As used herein, the unit of measurement „U“ and/or „international units“ refers to the blood glucose lowering activity of insulin and is defined (according to the World Health Organization, WHO) as follows: 1 U corresponds to the amount of highly purified insulin (as defined by the WHO) which is sufficient to lower the blood glucose level of a rabbit (having a body weight of 2 – 2.5 kg) to 50 mg / 100 mL within 1 hour and to 40 mg / 100 mL within 2 hours. For human insulin, 1 U corresponds to approximately 35 µg (Lill, Pharmazie in unserer Zeit, No. 1, pp. 56-61, 2001). For insulin aspart, 100 U correspond to 3.5 mg (product information NovoRapid®). For insulin lispro, 100 U correspond to 3.5 mg (product information Humalog®). For insulin glulisine, 100 U

correspond to 3.49 mg (product information Apidra® cartridges). For insulin determir, 100 U correspond to 14.2 mg (product information Levemir®). For insulin glargin, 100 U correspond to 3.64 mg (product information Lantus®).

Further embodiments of the present invention include the following:

In one aspect, the invention provides a pharmaceutical formulation comprising (a). at least one analogue and/or derivative of insulin; and (b). Zn(II); and (c). optionally protamine; wherein the pharmaceutical formulation is free of glycerol.

In one aspect, the pharmaceutical formulation of to the invention is an aqueous pharmaceutical formulation.

In one aspect, the pharmaceutical formulation of the invention has a pH value in the range from 6.0 to 9.0.

In one aspect, the pharmaceutical formulation of to the invention has a pH value in the range from 7.0 to 7.8.

In one aspect, the pharmaceutical formulation of the invention comprises an analogue of insulin selected from the group consisting of insulin aspart, insulin lispro and insulin glulisine.

In one aspect, the pharmaceutical formulation of the invention comprises a derivative of insulin selected from the group comprising insulin detemir and/or insulin degludec.

In one aspect, the pharmaceutical formulation of the invention comprises an analogue and/or derivative of insulin, wherein the analogue and/or derivative of insulin is present in a concentration from 10 U/mL to 1000 U/mL.

In one aspect, the pharmaceutical formulation of the invention comprises Zn(II) which is present in a concentration from 0.0100 to 0.0600 mg / 100 U of the analogue and/or derivative of insulin.

In one aspect, the pharmaceutical formulation of the invention further comprises sodium.

In one aspect, the pharmaceutical formulation of the invention comprises sodium chloride which is present in a concentration from 5.0 to 15 mg/mL.

In one aspect, the pharmaceutical formulation of the invention comprises sodium chloride which is present in a concentration from 5.6 to 6.8 mg/mL.

In one aspect, the pharmaceutical formulation of the invention comprises protamine which is present in a concentration from 0.1 to 0.5 mg/mL.

In one aspect, the pharmaceutical formulation of the invention comprises one or more further active pharmaceutical ingredients.

In one aspect, the pharmaceutical formulation of the invention comprises one or more further active pharmaceutical ingredients, wherein the further active pharmaceutical ingredient is an antidiabetic agent.

In one aspect, the pharmaceutical formulation of the invention comprises one or more further active pharmaceutical ingredients, wherein the further active pharmaceutical ingredient is an antidiabetic agent selected from the group consisting of: (a). a GLP-1 receptor agonist; (b). a dual GLP-1 receptor/glucagon receptor agonist; (c). human FGF-21; (d). an FGF-21 analogue; (e). an FGF-21 derivative; (f). insulin; (g). human insulin; (h). an analogue of insulin; and (i). a derivative of insulin.

In one aspect, the pharmaceutical formulation of the invention comprises more than one analogue and/or derivative of insulin, wherein one analogue and/or derivative of insulin is a fast acting insulin and one analogue and/or derivative of insulin is a long acting insulin.

In one aspect, the pharmaceutical formulation of the invention comprises more than one analogue and/or derivative of insulin, wherein one analogue and/or derivative of insulin is a fast acting insulin and one analogue and/or derivative of insulin is a long acting insulin, wherein the fast acting insulin is one or more insulin selected from the group consisting of insulin aspart, insulin lispro and insulin glulisine and wherein the long acting insulin is one or more insulin selected from the group consisting of insulin detemir and insulin degludec.

In one aspect, the pharmaceutical formulation of the invention consists of: (a). 3.5 mg/mL insulin aspart; (b). 1.72 mg/mL metacresol; (c). 1.50 mg/mL phenol; (d). 0.0196 mg/mL Zn(II); (e). 6.2 mg/mL sodium chloride; (f). 1.88 mg/mL Na_2HPO_4 x 7 H_2O; (g). sodium hydroxide and/or hydrochloric acid to adjust the pH to 7.4; and (i). water.

In one aspect, the pharmaceutical formulation of the invention consists of: (a). 3.5 mg/mL insulin aspart; (b). 1.72 mg/mL metacresol; (c). 1.50 mg/mL phenol; (d). 0.0196 mg/mL Zn(II); (e). 6.2 mg/mL sodium chloride; (f). 1.88 mg/mL Na_2HPO_4 x 7 H_2O; (g). from 0.1 mg/mL to 0.5 mg/mL protamine; (h). sodium hydroxide and/or hydrochloric acid to adjust the pH to a pH in the range from 7.1 to 7.6; and (i). water.

In one aspect, the invention provides a process for preparing the pharmaceutical formulation of the invention, wherein the components are mixed together in the form of a solution or suspension, the pH is adjusted to reach the desired pH and water is added to reach the final volume.

In one aspect, the invention provides a kit comprising one or more separate packages of (a). the pharmaceutical formulation of the invention; and (b). a medical device.

In one aspect, the invention provides a kit comprising one or more separate packages of (a). the pharmaceutical formulation of the invention; and (b). at least one further active pharmaceutical ingredient; (c). and optionally a medical device.

In one aspect, the kit of the invention comprises a further active pharmaceutical ingredient which is an antidiabetic agent.

In one aspect, the kit of the invention comprises a further active pharmaceutical ingredient which is an antidiabetic agent selected from the group consisting of: (a). a GLP-1 receptor agonist; (b).a dual GLP-1 receptor/glucagon receptor agonist; (c). human FGF-21; (d). a FGF-21 analogue; (e). a FGF-21 derivative; (f). insulin; (g). human insulin; (h). an analogue of insulin; and (i). a derivative of insulin.

In one aspect, the kit of the invention comprises more than one analogue and/or derivative of insulin, wherein one analogue and/or derivative of insulin is a fast acting insulin and one analogue and/or derivative of insulin is a long acting insulin.

In one aspect, the kit of the present invention, comprises more than one analogue and/or derivative of insulin, wherein one analogue and/or derivative of insulin is a fast acting insulin and one analogue and/or derivative of insulin is a long acting insulin wherein the fast acting insulin is selected from the group consisting of insulin aspart, insulin lispro and insulin glulisine and wherein the long acting insulin is selected from the group consisting of insulin glargin, insulin detemir and insulin degludec.

In one aspect, the invention provides a pharmaceutical formulation or kit for use in the treatment of diabetes mellitus.

In one aspect, the invention provides a pharmaceutical formulation or kit for use in the treatment of hyperglycemia.

In one aspect, the invention provides a pharmaceutical formulation or kit for use in lowering blood glucose level.

In one aspect, the invention provides a method of treating diabetes mellitus in a subject in need thereof comprising administering the pharmaceutical formulation of the invention.

In one aspect, the invention provides a method of treating hyperglycemia in a subject in need thereof comprising administering the pharmaceutical formulation of the invention.

In one aspect, the invention provides a method of lowering blood glucose levels in a subject in need thereof comprising administering the pharmaceutical formulation of the invention.

In one aspect, the invention provides a medical device for administering the pharmaceutical formulation of the invention to an animal and/or human.

The present invention is illustrated by the following Examples. However, it should be understood that the present invention is not limited to the specific details of these examples.

EXAMPLES

Example 1

Manufacturing process

(a) Zinc Chloride Solution

Zinc Chloride Solution was prepared by dissolving 2.00 g zinc chloride in water for injection and by filling up with water for injection to final volume of 1000 mL.

(b) Solution A

The final composition of Solution A is given in Table 1:

Table 1: Composition of Solution A

	Excipient	Composition per 200 mL	Composition per 2000 mL
1.	di-Natriumhydrogen-phosphat * 7 H_2O	1.88 g	18.8 g
2.	Sodium chloride	6.2 g	62.0 g
3.	Phenol	1.5 g	15.0 g
4.	m-Cresol	1.72 g	17.2 g
5.	Sodium hydroxide solution	ad pH 8.65	ad pH 8.65
6.	Hydrochloric acid	ad pH 8.65	ad pH 8.65
7.	Water for Injection	ad 200 mL = 201.5g	ad 2000 mL = 2015 g

Solution A was prepared as described in the following:

1. It was started with approximately 1000 g water for injection.
2. 18.8 g di-Natriumhydrogen-phosphat * 7 H_2O , 62.00 g sodium chloride, 15.0 g phenol and 17.2 g m-cresol were dissolved while stirring constantly.
3. Solution was filled up to approximately 1800 g with water for injection.
4. Solution was stirred for approximately 15 min using a magnetic stirrer.

5. pH was checked (pH should be 8.65). If pH value is not 8.65, the pH was adjusted to said range using hydrochloric acid 0.03 N or sodium hydroxide solution 1 N.
6. Solution was filled up to 2015 g (=1000 mL) with water for injection.

(c) Final Solution

The final composition of Final Solution is given in Table 2:

Table 2: Composition of Final Solution

	Excipient	Composition per mL	Composition per 1000 mL	Composition per 2000 mL
1.	Insulin aspart	3.5 mg	3.5 g	7.0 g
2.	Zn(II)	19.6 µg	0.0196 g	0.0392 g
3.	di-Natriumhydrogen-phosphat * 7 H_2O	1.88 mg	1.88 g	3.76 g
4.	Sodium chloride	6.20 mg	6.20 g	12.40 g
5.	Phenol	1.50 mg	1.50 g	3.0 g
6.	m-Cresol	1.72 mg	1.72 g	3.44 g
7.	Sodium hydroxide	ad pH 7.4	ad pH 7.4	ad pH 7.4
8.	Hydrochloric acid	ad pH 7.4	ad pH 7.4	ad pH 7.4
9.	Water for Injection	ad 1 mL = 1.005 g	ad 1000 mL = 1005 g	ad 2000 mL = 2010 g

Final Solution was prepared as described in the following:

1. It was started with 300 mL water for injection.
2. 7.0 g insulin aspart was added to the 300 mL water for injection while stirring constantly (a suspension of insulin aspart in water for injection is formed).
3. pH value was checked.
4. pH value was changed to approximately 3.1 to 3.2 by adding hydrochloric acid 0.03 N or sodium hydroxide solution 0.02 N to dissolve the insulin aspart.
5. Solution was stirred for approximately 15 min using a magnetic stirrer.
6. 41 mL Zinc Chloride Solution was added to the solution while stirring constantly.
7. Solution was filled up to 600 g with water for injection.
8. 400 mL Solution A was added slowly and carefully while stirring constantly.
9. pH was adjusted to 7.4 (range 7.2 to 7.6) using hydrochloric acid 0.03 N or sodium hydroxide solution 0.02 N.

10. Solution was filled up to 2010 g with water for injection (corresponds to 100 % of the Final Solution).

Quality control: Final solution was a clear and uncoloured solution, showed a pH value of 7.4 (plus/minus 0.2; at 20-25°C).

The Final Solution was applied to sterile filtration using ´Sartopore Minisart high flow´ filter (filter material: polyethersulfone; pore size: 0.2 µm; supplier: Sartorius).

The Final Solution after sterile filtration was a clear and uncoloured solution and showed an osmolarity of 260 mOsmol/kg (plus/minus 30).

The Final Solution after sterile filtration was filled into appropriate vials (volume: 5 and 10 mL; 13 mm; clear glas; glas type 1).

The vials –containing the Final Solution after sterile filtration– were stored between +2°C and + 8°C and protected from light.

Example 2

Control of the formulation

(a) Analytical procedures

Tests are carried out using compendial analytical test methods, where applicable. The quality control concept has been established taking into account the cGMP requirements as well as the current status of the ICH process.

The non-compendial and chromatographic analytical procedures used to control the formulation are summarized in the following:

Description

Visually examine a number of containers for conformance to the acceptance criteria.

Identification (HPLC)

The identity of the active ingredient is ensured by comparing the retention time of the drug formulation sample with the retention time of the reference standard using a reversed phase HPLC method. The method is also used for the determination of assay of the active ingredient, for the determination of the related compounds and impurities, and for quantifying the preservatives m-cresol and phenol.

Assay (HPLC)

The test is carried out by reverse phase liquid chromatography (HPLC). The method is also used for the identification, the determination of assay of the active ingredient, for the determination of the related compounds and impurities, and for quantifying the preservatives m-cresol and phenol. Column: Lichrosorb RP18, particle size 5 µm, pore size 100 Å (250 mm × 4.0 mm), thermostated at +35 °C. Autosampler: Thermostated at ≤+8 °C. Mobile phase A: Sodium sulfate solved in water, 14 g/mL, adjusted with phosphoric acid and sodium hydroxide to a pH of 3.4. Mobile phase B: Water/acetonitrile (50:50 v/v). Gradient is shown in Table 3.

Table 3: HPLC gradient

Time [min]	Mobile phase A [%]	Mobile phase B [%]
0 to 42	57.7	42.3
42 to 47 linear to	20	80
47 to 52	20	80
52 to 53 linear to	57.7	42.3
53 to 60 equilibration	57.7	42.5

Flow rate: 1.0 mL/min. Injection volume: 10 µL. Detection: 214 nm (for the active ingredient) and 260 nm (for m-cresol and phenol). Typical run time: 60 min.

Assay of the active ingredient, m-cresol and phenol are calculated by external standardization. Impurities are calculated using the peak area percent method.

Test solution: The formulation is used without any dilution or further treatment.

Related compounds and impurities (HPLC)

The same chromatographic conditions as for “Assay (HPLC)” are used for the determination of related compounds and impurities. Related compounds and Impurities are calculated using the peak area percent method.

High molecular weight proteins (HMWPs)

The high molecular weight proteins are determined using high pressure size exclusion chromatography (HPSEC). Column: Waters Insulin HMWP, particle size 5-10 µm, pore size 12 – 12.5 nm (300 mm × 7.8 mm), thermostated at room temperature. Autosampler: thermostated at ≤+8°C. Mobile phase: 650 mL of arginine solution (1 g/L) is mixed with 200 mL of acetonitrile and 150 mL of glacial acetic acid. Isocratic elution Flow rate: 1.0 mL/min. Injection volume: 100 µL. Detection: 276 nm. Typical run time: 35 min.

HMWPs are calculated using the peak area percent method. Test solution: The formulation is used without any dilution or further treatment.

Antimicrobial preservative assay

The same chromatographic conditions as for “Assay (HPLC)” are used for the determination of assay of m-Cresol and of phenol m-cresol and phenol are calculated by external standardization.

(b) Validation of analytical procedures

The HPLC analytical procedure for the formulation for the determination of identification, assay, and related compounds and impurities was validated to demonstrate specificity, linearity, limit of detection and limit of quantification, accuracy, precision and range.

(c) Justification of the acceptance criteria

Tests and acceptance criteria, as previously presented, were selected based on ICH Q6B and on published monographs, analytical results obtained, precision of procedures used, Pharmacopoeial and/or regulatory guidelines, and are in agreement with the standard limits at this stage of development.

Example 3

Stability of the formulation

(a) Stability of the formulation

Stability studies for the formulation were initiated according to the stability protocol summary described in the following table. The composition and manufacturing method of the stability

batches are representative of the material. The stability profile was assessed for storage under long term, accelerated, and stress testing conditions according to ICH guidelines. Samples were packed and stored in glass vials with flanged cap with inserted disc and flip-off lid. The stability data obtained using this packaging material are representative for the preliminary shelf life and storage direction for both packaging configurations (10 mL glass vials and 3 mL cartridges). Up to now, 6 months stability data are available from ongoing stability studies of the formulation.

Table 4: Storage Conditions

Storage Condition	Sampling Intervals	Container
Long Term		
+5°C±3°C	1, 3 months	10 mL vials
Accelerated		
+25°C±2°C/60%±5% RH	1, and 3months	10 mL vials
Stress		
+40°C±5°C/75%±5% RH	1 month	10 mL vials
Photostability		
Sun test according to ICH guidelines*	1 day	10 mL vials
Indoor light**	14 days	10 mL vials
* Overall illumination of not less than 1.2 million lux hours and an integrated near ultraviolet energy of not less than 200 watt hours/m2. A dark control sample is stored under the same conditions to eliminate any effects due to local temperature changes **Variolux, Heraeus, standard fluorescent tubes, GE-Lightening, Type F40/33, irradiance approximately 8 W/m2, 2000 Lux. A dark control sample is stored under the same conditions to evaluate any effects due to local temperature changes		

The following tests were performed during stability testing: appearance, assay, related impurities, high molecular weight proteins, pH, assay of antimicrobial preservatives (m-cresol and phenol), content of zinc (Zn(II)). The investigations on physical and chemical properties after 3 months of storage at the long term storage condition of +5°C confirm the stability of the formulation when stored at the recommended storage condition. Only very slight changes of the related impurities could be observed.

When stored at accelerated conditions (3 months at +25°C/60%RH) the related impurities and high molecular weight proteins increased, however stayed well within the current acceptance limit. When stored at accelerated conditions (1 month at +40°C/75%RH) one of the related impurities

increased above the acceptance criterion. The content of the active ingredient, m-cresol and phenol remained basically unchanged under accelerated conditions.

When stored exposed to light (sun test according to ICH guidelines for 1 day and indoor light for 14 days) the related impurities (total of other impurities) and high molecular weight proteins increased above the acceptance criteria. The content of the active ingredient, m-cresol and phenol, remained basically unchanged after photostability testing.

Due to the present results of the stability studies of the formulation, the chemical and physical stability of the formulation can be confirmed.

Tables 5-8 show the long term stability results, wherein batch no. "_0025" refers to a formulation according to the present invention.

The stability of the formulation as presently claimed shows an excellent chemical and physical stability which qualifies said aqueous pharmaceutical formulation as medicinal product having a defined shelf life.

Table 5: Long term stability +5°C batch _0025

Storage condition:	+5°C±3°C			
Test item	**Acceptance criteria for clinical trials**	**Time**		
		Initial results	**1 month**	**3 months**
Appearance of solution (visual)				
- Clarity	Monitoring	< I (water clear)	< I (water clear)	< I (clear)
- Color	Monitoring	B9	B9	B9
Assay in insulin aspart units (HPLC)	90.0 insulin aspart units/mL to 110.0 insulin aspart units/mL	107.1 insulin aspart units/mL (3.75 mg/mL)	105.4 insulin aspart units/mL (3.69 mg/mL)	105.7 insulin aspart units/mL (3.70 mg/mL)
Related Compounds(HPLC)				
- B28isoAsp insulin aspart	≤2.5%	0.26%	0.24%	0.31%
- Total of A21Asp insulin aspart, B3Asp insulin aspart and B3isoAsp insulin aspart	≤5.0%	1.27%	1.21%	1.22%
- Any other unspecified, unidentified impurity	≤2.0%	0.41%	0.48%	0.29%
- Total of other impurities	≤3.5%	0.53%	0.74%	0.41%
High molecular weight proteins (HPSEC)	≤1.5%	0.19%	0.25%	0.21%
pH	Between 7.0 to 7.8	7.40	7.38	7.37
Assay m-cresol	1.55 mg/mL to 1.89 mg/mL (90.0% to 110.0% of label claim)	1.76 mg/mL (102.3%)	1.79 mg/mL (104.1%)	1.70 mg/mL (98.8%)
Assay phenol	1.35 mg/mL to 1.65 mg/mL (90.0% to 110.0% of label claim)	1.48 mg/mL (98.6%)	1.50 mg/mL (100.0%)	1.49 mg/mL (99.3%)
Zinc (Zn(II)) (AAS)	<40 µg per 100 units insulin aspart	19.1 µg per 100 units insulin aspart (20.5 µg/mL)	Not tested	Not tested

Table 6: Accelerated stability +25°C/60% RH batch _0025

Storage condition:	+25°C±2°C / 60%±5%RH			
Test item	**Acceptance criteria for clinical trials**	**Time**		
		Initial results	**1 month**	**3 months**
Appearance of solution (visual)				
- Clarity	Monitoring	< I (water clear)	< I (water clear)	< I (clear)
- Color	Monitoring	B9	B9	B9
Assay in insulin aspart units (HPLC)	90.0 insulin aspart units/mL to 110.0 insulin aspart units/mL	107.1 insulin aspart units/mL (3.75 mg/mL)	103.7 insulin aspart units/mL (3.63 mg/mL)	102.0 insulin aspart units/mL (3.57 mg/mL)
Related Compounds(HPLC)				
- B28isoAsp insulin aspart	≤2.5%	0.26%	0.87%	2.21%
- Total of A21Asp insulin aspart, B3Asp insulin aspart and B3isoAsp insulin aspart	≤5.0%	1.27%	1.48%	2.26%
- Any other unspecified, unidentified impurity	≤2.0%	0.41%	0.57%	0.63%
- Total of other impurities	≤3.5%	0.53%	0.79%	0.94%
High molecular weight proteins (HPSEC)	≤1.5%	0.19%	0.29%	0.51%
pH	Between 7.0 to 7.8	7.40	7.38	7.37
Assay m-cresol	1.55 mg/mL to 1.89 mg/mL (90.0% to 110.0% of label claim)	1.76 mg/mL (102.3%)	1.78 mg/mL (103.5%)	1.69 mg/mL (98.3%)
Assay phenol	1.35 mg/mL to 1.65 mg/mL (90.0% to 110.0% of label claim)	1.48 mg/mL (98.6%)	1.49 mg/mL (99.3%)	1.49 mg/mL (99.3%)
Zinc (Zn(II)) (AAS)	<40 µg per 100 units insulin aspart	19.1 µg per 100 units insulin aspart (20.5 µg/mL)	Not tested	Not tested

Table 7: Accelerated stability +40°C/75% RH batch _0025

Storage condition:	+40°C±2°C / 75%±5%RH		
Test item	**Acceptance criteria for clinical trials**	**Time**	
		Initial results	**1 month**
Appearance of solution (visual)			
- Clarity	Monitoring	< I (water clear)	< I (water clear)
- Color	Monitoring	B9	B9
Assay in insulin aspart units (HPLC)	90.0 insulin aspart units/mL to 110.0 insulin aspart units/mL	107.1 insulin aspart units/mL (3.75 mg/mL)	95.4 insulin aspart units/mL (3.34 mg/mL)
Related Compounds(HPLC)			
- B28isoAsp insulin aspart	≤2.5%	0.26%	4.46%
- Total of A21Asp insulin aspart, B3Asp insulin aspart and B3isoAsp insulin aspart	≤5.0%	1.27%	3.26%
- Any other unspecified, unidentified impurity	≤2.0%	0.41%	1.30%
- Total of other impurities	≤3.5%	0.53%	2.52%
High molecular weight proteins (HPSEC)	≤1.5%	0.19%	0.92%
pH	Between 7.0 to 7.8	7.40	7.40
Assay m-cresol	1.55 mg/mL to 1.89 mg/mL (90.0% to 110.0% of label claim)	1.76 mg/mL (102.3%)	1.77 mg/mL (102.9%)
Assay phenol	1.35 mg/mL to 1.65 mg/mL (90.0% to 110.0% of label claim)	1.48 mg/mL (98.6%)	1.49 mg/mL (99.3%)
Zinc (Zn(II)) (AAS)	<40 µg per 100 units insulin aspart	19.1 µg per 100 units insulin aspart (20.5 µg/mL)	21.0 µg per 100 units insulin aspart (20.0 µg/mL)

Table 8: Photostability Suntest batch _0025

Storage condition:	Suntest per ICH guideline and indoor light					
Test item	**Acceptance criteria for clinical trials**	**Time**				
			Sun test (ICH)		**Indoor light**	
		0	**Dark control**	**1 day**	**Dark control**	**14 days**
Appearance of solution (visual)						
- Clarity	Monitoring	< I (water clear)	< I (water clear)	< I (water clear)	< I (water clear)	< I (water clear)
- Color	Monitoring	B9	B9	B5	B9	B7
Assay in insulin aspart units (HPLC)	90.0 insulin aspart units/mL to 110.0 insulin aspart units/mL	107.1 insulin aspart units/mL (3.75 mg/mL)	105.7 insulin aspart units/mL (3.70 mg/mL)	98.6 insulin aspart units/mL (3.45 mg/mL)	105.4 insulin aspart units/mL (3.69 mg/mL)	100.5 insulin aspart units/mL (3.52 mg/mL)
Related Compounds(HPLC)						
- B28isoAsp insulin aspart	≤2.5%	0.26%	0.26%	0.25%	0.54%	0.53%
- Total of A21Asp insulin aspart, B3Asp insulin aspart and B3isoAsp insulin aspart	≤5.0%	1.27%	1.00%	1.02%	1.35%	1.76%
- Any other unspecified, unidentified impurity	≤2.0%	0.41%	0.22%	0.94%	0.39%	0.77%
- Total of other impurities	≤3.5%	0.53%	0.89%	5.72%	0.84%	3.83%
High molecular weight proteins (HPSEC)	≤1.5%	0.19%	0.20%	3.27%	0.24%	2.91%
pH	Between 7.0 to 7.8	7.40	7.41	7.39	7.39	7.39
Assay m-cresol	1.55 mg/mL to 1.89 mg/mL (90.0% to 110.0% of label claim)	1.76 mg/mL (102.3%)	1.74 mg/mL (101.2%)	1.72 mg/mL (100.0%)	1.78 mg/mL (103.5%)	1.76 mg/mL (102.3%)
Assay phenol	1.35 mg/mL to 1.65 mg/mL (90.0% to 110.0% of label claim)	1.48 mg/mL (98.6%)	1.48 mg/mL (98.7%)	1.47 mg/mL (98.0%)	1.51 mg/mL (100.7%)	1.51 mg/mL (100.7%)
Zinc (Zn(II)) (AAS)	<40 µg per 100 units insulin aspart	19.1 µg per 100 units insulin aspart (20.5 µg/mL)	19.2 µg per 100 units insulin aspart (20.3 µg/mL)	20.2 µg per 100 units insulin aspart (19.9 µg/mL)	19.0 µg per 100 units insulin aspart (20.0 µg/mL)	20.6 µg per 100 units insulin aspart (20.2 µg/mL)

Claims

1. A pharmaceutical formulation comprising
(a). at least one analogue and/or derivative of insulin; and
(b). Zn(II); and
(c). optionally protamine;
wherein the pharmaceutical formulation is free of glycerol.

2. The pharmaceutical formulation according to claim 1, wherein the pharmaceutical formulation is an aqueous pharmaceutical formulation.

3. The pharmaceutical formulation according to claim 1 or 2, wherein the pharmaceutical formulation has a pH value in the range from 6.0 to 9.0.

4. The pharmaceutical formulation according to claim 3, wherein
the pharmaceutical formulation has a pH value in the range from 7.0 to 7.8.

5. The pharmaceutical formulation as claimed in one or more of claims 1 to 4, wherein the analogue of insulin is selected from the group consisting of insulin aspart, insulin lispro and insulin glulisine.

6. The pharmaceutical formulation as claimed in one or more of claims 1 to 4, wherein the derivative of insulin is selected from the group comprising insulin detemir and/or insulin degludec.

7. The pharmaceutical formulation as claimed in one or more of claims 1 to 6, wherein the analogue and/or derivative of insulin is present in a concentration from 10 U/mL to 1000 U/mL.

8. The pharmaceutical formulation as claimed in one or more of claims 1 to 7, wherein Zn(II) is present in a concentration from 0.0100 to 0.0600 mg / 100 U of the analogue and/or derivative of insulin.

9. The pharmaceutical formulation as claimed in one or more of claims 1 to 8, wherein said pharmaceutical formulation further comprises sodium chloride.

10. The pharmaceutical formulation as claimed according to claim 9, wherein sodium chloride is present in a concentration from 5.0 to 15 mg/mL.

11. The pharmaceutical formulation according to claim 9 or 10 , wherein sodium chloride is present in a concentration from 5.6 to 6.8 mg/mL.

12. The pharmaceutical formulation as claimed in one or more of claims 1 to 10, wherein protamine is present in a concentration from 0.1 to 0.5 mg/mL.

13. The pharmaceutical formulation as claimed in one or more of claims 1 to 11, wherein the pharmaceutical formulation comprises one or more further active pharmaceutical ingredients.

14. The pharmaceutical formulation according to claim 12, wherein the further active pharmaceutical ingredient is an antidiabetic agent.

15. The pharmaceutical formulation according to claim 12 or 13, wherein the further active pharmaceutical ingredient is an antidiabetic agent selected from the group consisting of:
(a). a GLP-1 receptor agonist;
(b). a dual GLP-1 receptor/glucagon receptor agonist;
(c). human FGF-21;
(d). an FGF-21 analogue;
(e). an FGF-21 derivative;
(f). insulin;
(g). human insulin;
(h). an analogue of insulin; and
(i). a derivative of insulin.

16. The pharmaceutical formulation as claimed in one or more of claims 1 to 14, wherein the pharmaceutical formulation comprises more than one analogue and/or derivative of insulin,

wherein one analogue and/or derivative of insulin is a fast acting insulin and one analogue and/or derivative of insulin is a long acting insulin.

17. The pharmaceutical formulation according to claim 15, wherein the fast acting insulin is one or more insulin selected from the group consisting of insulin aspart, insulin lispro and insulin glulisine and wherein the long acting insulin is one or more insulin selected from the group consisting of insulin detemir and insulin degludec.

18. The pharmaceutical formulation as claimed in one or more of claims 1 to 16, wherein the pharmaceutical formulation consists of:
(a). 3.5 mg/mL insulin aspart;
(b). 1.72 mg/mL metacresol;
(c). 1.50 mg/mL phenol;
(d). 0.0196 mg/mL Zn(II);
(e). 6.2 mg/mL sodium chloride;
(f). 1.88 mg/mL Na_2HPO_4 x 7 H_2O;
(g). sodium hydroxide and/or hydrochloric acid to adjust the pH to 7.4; and
(i). water.

19. The pharmaceutical formulation as claimed in one or more of claims 1 to 16, wherein the pharmaceutical formulation consists of:
(a). 3.5 mg/mL insulin aspart;
(b). 1.72 mg/mL metacresol;
(c). 1.50 mg/mL phenol;
(d). 0.0196 mg/mL Zn(II);
(e). 6.2 mg/mL sodium chloride;
(f). 1.88 mg/mL Na_2HPO_4 x 7 H_2O;
(g). from 0.1 mg/mL to 0.5 mg/mL protamine;
(h). sodium hydroxide and/or hydrochloric acid to adjust the pH to a pH in the range from 7.1 to 7.6; and
(i). water.

20. A process for preparing the pharmaceutical formulation according to claim 1, wherein the components are mixed together in the form of an solution or suspension, the pH is adjusted to reach the desired pH and water is added to reach the final volume.

21. A kit comprising one or more separate packages of
(a). the pharmaceutical formulation as claimed in one or more of claims 1 to 18; and
(b). a medical device.

22. A kit comprising one or more separate packages of
(a). the pharmaceutical formulation as claimed in one or more of claims 1 to 18; and
(b). at least one further active pharmaceutical ingredient;
(c). and optionally a medical device.

23. The kit according to claim 20 or 21, wherein the further active pharmaceutical ingredient is an antidiabetic agent.

24. The kit as claimed in one or more of claims 20 to 22, wherein the further active pharmaceutical ingredient is an antidiabetic agent selected from the group consisting of:
(a). a GLP-1 receptor agonist;
(b). a dual GLP-1 receptor/glucagon receptor agonist;
(c). human FGF-21;
(d). a FGF-21 analogue;
(e). a FGF-21 derivative;
(f). insulin;
(g). human insulin;
(h). an analogue of insulin; and
(i). a derivative of insulin.

25. The kit as claimed in one or more of claims 20 to 23, wherein the kit comprises more than one analogue and/or derivative of insulin, wherein one analogue and/or derivative of insulin is a fast acting insulin and one analogue and/or derivative of insulin is a long acting insulin.

26. The kit according to claim 24, wherein the fast acting insulin is selected from the group consisting of insulin aspart, insulin lispro and insulin glulisine and wherein the long acting insulin is selected from the group consisting of insulin glargin, insulin detemir and insulin degludec.

27. The pharmaceutical formulation as claimed in one or more of claims 1 to 18 or the kit as claimed in one or more of claims 20 to 25 for use in the treatment of diabetes mellitus.

28. The pharmaceutical formulation as claimed in one or more of claims 1 to 18 or the kit as claimed in one or more of claims 20 to 25 for use in the treatment of hyperglycemia.

29. The pharmaceutical formulation as claimed in one or more of claims 1 to 18 or the kit as claimed in one or more of claims 20 to 25 for use in lowering blood glucose level.

30. A method of treating diabetes mellitus in a subject in need thereof comprising administering the pharmaceutical formulation of any one of claims 1 to 18.

31. A method of treating hyperglycemia in a subject in need thereof comprising administering the pharmaceutical formulation of any one of claims 1 to 18.

32. A method of lowering blood glucose levels in a subject in need thereof comprising administering the pharmaceutical formulation of any one of claims 1 to 18.

33. Medical device for administering the pharmaceutical formulation as claimed in one or more of claims 1 to 18 to an animal and/or human.