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(54) Benevnelse **AQUEOUS PREPARATIONS COMPRISING METHIONINE**

(56) Anførte publikasjoner

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WO-A1-2009/048959
WO-A2-2008/124522
WO-A2-2008/133908
WO-A2-2009/087081
WO-A2-2009/134380
US-A1- 2006 093 576
US-B2- 6 852 694

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Description

The invention relates to an aqueous pharmaceutical formulation with an insulin, insulin analogue or insulin derivative, and methionine; and also to its preparation, use for treating diabetes mellitus, and to a medicament for treating diabetes mellitus.

An increasing number of people around the world suffer from diabetes mellitus. Many of them are what are called type I diabetics, for whom replacement of the deficient endocrine insulin secretion is the only possible therapy at present. Those affected are dependent on insulin injections for life, usually several times a day. Type II diabetes contrasts with type I diabetes in that there is not always a deficiency of insulin, but in a large number of cases, especially at the advanced stage, treatment with insulin, where appropriate in combination with an oral antidiabetic, is considered the most advantageous form of therapy.

In healthy individuals, release of insulin by the pancreas is strictly coupled to the blood glucose concentration. Elevated blood glucose levels, like those occurring after meals, are quickly compensated by a corresponding rise in insulin secretion. In the fasting state, the plasma insulin level falls to a base line value which is sufficient to ensure a continuous supply of glucose to insulin-sensitive organs and tissues, and to keep hepatic glucose production low in the night. The replacement of the endogenous insulin secretion by exogenous, usually subcutaneous administration of insulin does not in general come close to the above-described quality of the physiological regulation of blood glucose. Frequently there are instances of blood glucose being thrown off-track, either upwardly or downwardly, and in their most severe forms these instances may be life-threatening. In addition, however, blood glucose levels which are elevated over years, without initial symptoms, constitute a considerable health risk. The large-scale DCCT study in the USA (The Diabetes Control and Complications Trial Research Group (1993), N. Engl. J. Med. 329, 977-986) showed

unambiguously that chronically elevated blood glucose levels are responsible for the development of late diabetic complications. Late diabetic complications are microvascular and macrovascular damage which is manifested in certain circumstances as retinopathy, nephropathy, or neuropathy, and leads to blindness, renal failure, and loss of extremities, and, in addition, is associated with an increased risk of cardiovascular disorders. From this it can be inferred that an improved therapy of diabetes must be aimed primarily at keeping blood glucose as closely as possible within the physiological range. According to the concept of intensified insulin therapy, this is to be achieved by means of injections, several times a day, of fast-acting and slow-acting insulin preparations. Fast-acting formulations are given at meal times, in order to compensate the postprandial rise in blood glucose. Slow-acting basal insulins are intended to ensure the basic supply of insulin, especially during the night, without leading to hypoglycemia.

Insulin is a polypeptide composed of 51 amino acids which are divided between two amino acid chains: the A chain, with 21 amino acids, and the B chain, with 30 amino acids. The chains are linked together by two disulfide bridges. Insulin preparations have been employed for many years in diabetes therapy. Such preparations use not only naturally occurring insulins but also, more recently, insulin derivatives and insulin analogues.

Insulin analogues are analogues of naturally occurring insulins, namely human insulin or animal insulins, which differ by replacement of at least one naturally occurring amino acid residue by other amino acids and/or by addition/deletion of at least one amino acid residue, from the corresponding, otherwise identical, naturally occurring insulin. The amino acids in question may also be amino acids which do not occur naturally.

Insulin derivatives are derivatives of naturally occurring insulin or an insulin analogue which are obtained by chemical modification. The chemical modification may consist, for example, in the addition of one or more defined chemical groups to one or more amino acids. Generally speaking, the activity of

insulin derivatives and insulin analogues is somewhat altered as compared with human insulin.

Insulin analogues with an accelerated onset of action are described in EP 0 214 826, EP 0 375 437, and EP 0 678 522. EP 0 124 826 relates, among other things, to replacements of B27 and B28. EP 0 678 522 describes insulin analogues which have different amino acids in position B29, preferably proline, but not glutamic acid.
EP 0 375 437 encompasses insulin analogues with lysine or arginine at B28, which may also optionally be modified at B3 and/or A21.

EP 0 419 504 discloses insulin analogues which are protected from chemical modifications by modification of asparagine in B3 and of at least one further amino acid at positions A5, A15, A18 or A21.

Generally speaking, insulin derivatives and insulin analogues have a somewhat altered action as compared with human insulin.

US 6 852 694 discloses a stabilized insulin composition which comprises a mixture of insulin species. In this composition, heterodimeric complexes are formed from the two insulin species, these heterodimeric complexes having a greater stability than homodimeric complexes in compositions containing only one insulin species.

WO 2008/133908 discloses suspension formulations of insulinotropic peptides such as GLP-1 or exendin-4, for example. The suspension formulation comprises a particle formulation comprising an insulinotropic peptide, a disaccharide, methionine and a buffer, and a nonaqueous suspension medium which comprises one or more pyrrolidone polymers and one or more solvents.

US 2006/0093576 discloses a stabilized interleukin-2 formulation produced by contacting the interleukin-2 with an amino acid and a buffer. The amino acid is

selected from arginine, lysine, aspartic acid, and glutamic acid. The formulation may further comprise methionine in an amount to inhibit the oxidation of at least one methionine residue in the interleukin-2.

WO 99/62558 discloses a child nutrition formulation in powder or liquid form that has been supplemented with insulin in order to reduce the risk of the children becoming ill from diabetes.

WO 2009/048959 discloses fast-acting injectable insulin compositions. The formulation comprises a zinc chelator such as EDTA or EGTA, for example.

WO 2008/124522 discloses compositions comprising an insulin, a zinc chelator, and a solvent in combination with a GLP-1 mimetic or a GLP-1 analogue.

WO 92/00321 describes insulin analogues in which at least one amino acid in positions B1-B6 has been replaced by lysine or arginine. Such insulins, according to WO 92/00321, have an extended effect. A delayed effect is also exhibited by the insulin analogues described in EP-A 0 368 187. The concept of intensified insulin therapy attempts to reduce the risk to health by aiming for stable control of the blood sugar level by means of early administration of basal insulins. One example of a common basal insulin is the drug Lantus® (active ingredient: insulin glargine = Gly (A21), Arg (B31), Arg (B32) human insulin). Generally speaking, the aim in the development of new, improved basal insulins is to minimize the number of hypoglycemic events. An ideal basal insulin acts safely in each patient for at least 24 hours. Ideally, the onset of the insulin effect is delayed and has a fairly flat time/activity profile, thereby significantly minimizing the risk of short-term undersupply of sugar, and allowing administration even without food being taken beforehand. The supply of basal insulin is effective when the insulin activity goes on consistently for as long as possible, i.e., the body is supplied with a constant amount of insulin. As a result, the risk of hypoglycemic events is low, and patient-specific and day-specific variability are minimized. The pharmacokinetic profile of an ideal basal insulin,

then, ought to be characterized by a delayed onset of action and by a delayed action, i.e., a long-lasting and uniform action.

The preparations of naturally occurring insulins for insulin replacement that are present on the market differ in the origin of the insulin (e.g., bovine, porcine, human insulin) and also in their composition, and so the activity profile (onset and duration of action) may be affected. Through combination of different insulin products it is possible to obtain any of a very wide variety of activity profiles and to bring about very largely physiological blood sugar values. Recombinant DNA technology nowadays allows the preparation of modified insulins of this kind. They include insulin glargine (Gly(A21)-Arg(B31)-Arg(B32) human insulin), with an extended duration of action. Insulin glargine is injected in the form of a clear, acidic solution, and, on the basis of its dissolution properties is precipitated, in the physiological pH range of the subcutaneous tissue, as a stable hexamer association. Insulin glargine is injected once a day and is notable in comparison with other long-active insulins for its flat serum profile and the associated reduction in the risk of night hypoglycemias (Schubert-Zsilavecz et al., 2:125-130 (2001)). In contrast to preparations described to date, the specific preparation of insulin glargine that leads to the prolonged duration of action is characterized by a clear solution with an acidic pH. Specifically at acidic pH, however, insulins exhibit reduced stability and an increased tendency toward aggregation under thermal and physico-mechanical load, which may be manifested in the form of haze and precipitation (particle formation) (Brange et al., J. Ph. Sci 86:517-525 (1997)).

It has been found that such insulin analogues lead to the described desired basal time/activity profile, when the insulin analogues are characterized by the features that

• the B chain end is composed of an amidated basic amino acid residue such as lysine or arginine amide, i.e., in the amidated basic amino acid residue at the B chain end, the carboxyl group of the terminal amino acid is in its amidated form, and

• the N-terminal amino acid residue of the insulin A chain is a lysine or arginine residue, and

• the amino acid position A8 is occupied by a histidine residue, and

• the amino acid position A21 is occupied by a glycine residue, and

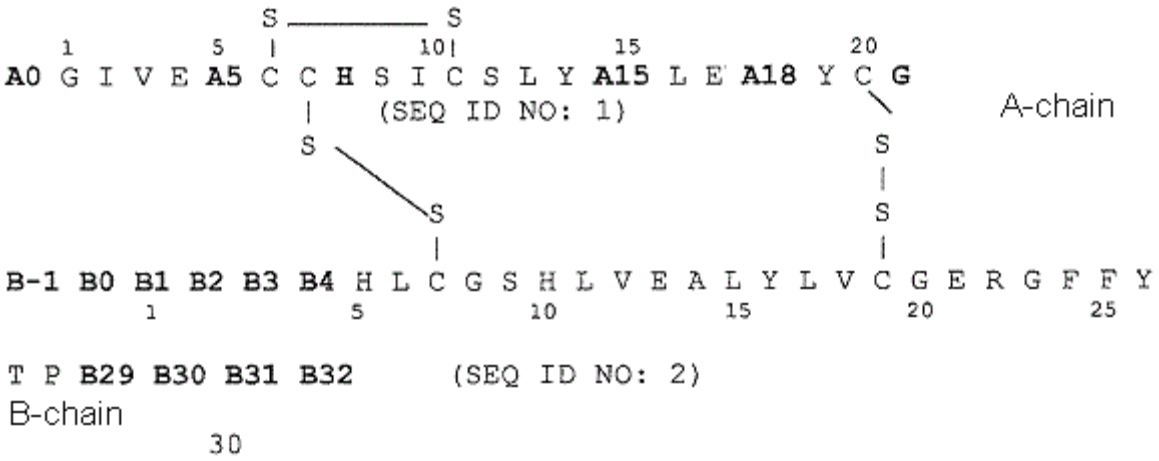
• there are two replacements of neutral amino acids by acidic amino acids, two additions of negatively charged amino acid residues, or one such replacement and one such addition, in each of positions A5, A15, A18, B-1, B0, B1, B2, B3, and B4.

Common to all aqueous formulations of insulins, insulin analogues, and insulin derivatives is that the stated proteins are not entirely stable chemically, but instead, as a function of the time, storage temperature, and movement to which the formulation is subject, and many more, there are a range of molecular processes that may occur, affecting the insulins, insulin analogues and insulin derivatives, that are deleterious to the quality of the formulation. One substance which impairs the chemical stability of insulins, insulin analogues, and insulin derivatives is oxygen, whose contact with the formulations in question is unavoidable, owing to its presence in the air - particularly in the case of formulations in packs for multiple administration. It is assumed that, among other things, it is the oxidative potential of oxygen that brings about the impairments in chemical stability.

It has now been found that, surprisingly, the addition of the amino acid methionine to formulations of insulins, insulin analogues, and insulin derivatives leads to an improved stability on the part of these proteins.

The invention accordingly provides an aqueous, pharmaceutical formulation comprising an insulin, insulin analogue or insulin derivative, or a pharmacologically tolerable salt thereof, and methionine, the pH of the formulation being 4.5 or less.

In the pharmaceutical formulation as described above, the insulin analogue is selected from the group containing Gly(A21), Arg(B31), Arg(B32) human insulin, Lys(B3), Glu(B29) human insulin, Asp(B28) human insulin, Lys(B28) Pro(B29) human insulin, Des(B30) human insulin and an insulin analogue of the formula I



where

A0 is Lys or Arg;

A5 is Asp, Gln or Glu;

A15 is Asp, Glu or Gln;

A18 is Asp, Glu or Asn;

B-1 is Asp, Glu or an amino group;

B0 is Asp, Glu or a chemical bond;

B1 is Asp, Glu or Phe;

B2 is Asp, Glu or Val;

B3 is Asp, Glu or Asn;

B4 is Asp, Glu or Gln;

B29 is Lys or a chemical bond;

B30 is Thr or a chemical bond;

B31 is Arg, Lys or a chemical bond;

B32 is Arg-amide, Lys-amide or an amino group,

where two amino acid residues of the group containing A5, A15, A18, B-1, B0, B1, B2, B3, and B4, simultaneously and independently of one another, are Asp or Glu, and the insulin analogue being selected in particular from a group containing:

Arg (A0), His (A8), Glu (A5), Asp (A18), Gly (A21), Arg (B31), Arg (B32) – NH_2 human insulin,

Arg (A0), His (A8), Glu (A5), Asp (A18), Gly (A21), Arg (B31), Lys (B32) – NH_2 human insulin,

Arg (A0), His (A8), Glu (A15), Asp (A18), Gly (A21), Arg (B31), Arg (B32) – NH_2 human insulin,

Arg (A0), His (A8), Glu (A15), Asp (A18), Gly (A21), Arg (B31), Lys (B32) – NH_2 human insulin,

Arg (A0), His (A8), Glu(A5), Glu (A15), Gly (A21), Arg (B31), Arg (B32) – NH_2 human insulin,

Arg (A0), His (A8), Glu (A5), Glu (A15), Gly (A21), Arg (B31), Lys (B32) – NH_2 human insulin,

Arg (A0), His(A8), Glu (A5), Gly (A21), Asp (B3), Arg (B31), Arg (B32) – NH_2 human insulin,

Arg (A0), His(A8), Glu (A5), Gly (A21), Asp (B3), Arg (B31), Lys (B32) – NH_2 human insulin,

Arg (A0), His (A8), Glu (A15), Gly (A21), Asp (B3), Arg (B31), Arg (B32) – NH_2 human insulin,

Arg (A0), His (A8), Glu (A15), Gly (A21), Asp (B3), Arg (B31), Lys (B32) – NH_2 human insulin,

Arg (A0), His (A8), Asp (A18), Gly (A21), Asp (B3), Arg (B31), Arg (B32) – NH_2 human insulin,

Arg (A0), His (A8), Asp (A18), Gly (A21), Asp (B3), Arg (B31), Lys (B32) – NH_2 human insulin,

Arg (A0), His(A8), Gly (A21), Asp (B3), Glu (B4), Arg (B31), Arg (B32) – NH_2 human insulin,

Arg (A0), His (A8), Gly (A21), Asp (B3), Glu (B4), Arg (B31), Lys (B32) – NH_2 human insulin,

Arg (A0), His (A8), Glu (A5), Gly (A21), Glu (B4), Arg (B31), Arg (B32) – NH_2 human insulin,

Arg (A0), His (A8), Glu (A5), Gly (A21), Glu (B4), Arg (B31), Lys (B32) – NH_2 human insulin,

Arg (A0), His (A8), Glu (A15), Gly (A21), Glu (B4), Arg (B31), Arg (B32) – NH_2 human insulin,

Arg (A0), His (A8), Glu (A15), Gly (A21), Glu (B4), Arg (B31), Lys (B32) – NH_2 human insulin,

Arg (A0), His (A8), Asp (A18), Gly (A21), Glu (B4), Arg (B31), Arg (B32) – NH_2 human insulin,

Arg (A0), His (A8), Asp (A18), Gly (A21), Glu (B4), Arg (B31), Lys (B32) – NH_2 human insulin,

Arg (A0), His (A8), Glu (A5), Gly (A21), Glu (B0), Arg (B31), Arg (B32) – NH_2 human insulin,

Arg (A0), His (A8), Glu (A5), Gly (A21), Glu (B0), Arg (B31), Lys (B32) – NH_2 human insulin,

Arg (A0), His (A8), Glu (A15), Gly (A21), Glu (B0), Arg (B31), Arg (B32) – NH_2 human insulin,

Arg (A0), His (A8), Glu (A15), Gly (A21), Glu (B0), Arg (B31), Lys (B32) – NH_2 human insulin,

Arg (A0), His (A8), Asp (A18), Gly (A21), Glu (B0), Arg (B31), Arg (B32) – NH_2 human insulin,

Arg (A0), His (A8), Asp (A18), Gly (A21), Glu (B0), Arg (B31), Lys (B32) – NH_2 human insulin,

Arg (A0), His (A8), Glu (A5), Gly (A21), Asp (B1), Arg (B31), Arg (B32) – NH_2 human insulin,

Arg (A0), His (A8), Glu (A5), Gly (A21), Asp (B1), Arg (B31), Lys (B32) – NH_2 human insulin,

Arg (A0), His (A8), Glu (A15), Gly (A21), Asp (B1), Arg (B31), Arg(B32) – NH_2 human insulin,

Arg (A0), His (A8), Glu (A15), Gly (A21), Asp (B1), Arg (B31), Lys (B32) – NH_2 human insulin,

Arg (A0), His (A8), Asp (A18), Gly (A21), Asp (B1), Arg (B31), Arg (B32) – NH_2 human insulin,

Arg (A0), His (A8), Asp (A18), Gly (A21), Asp (B1), Arg (B31), Lys (B32) – NH_2 human insulin,

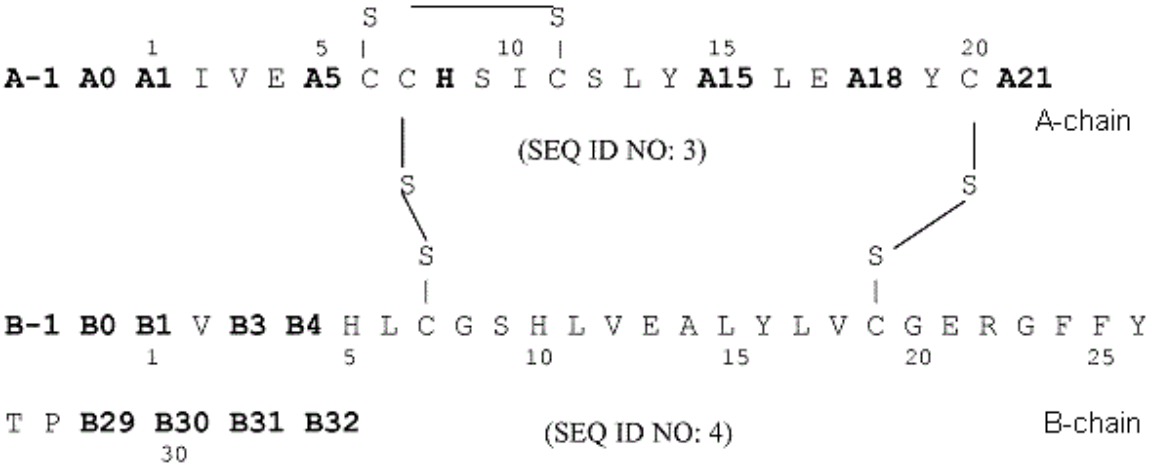
Arg (A0), His (A8), Gly (A21), Glu (B0), Asp (B1), Arg (B31), Arg (B32) – NH_2 human insulin,

Arg (A0), His (A8), Gly (A21), Glu (B0), Asp (B1), Arg (B31), Lys (B32) – NH_2 human insulin,

Arg (A0), His (A8), Asp (A18), Gly (A21), Asp (B3), Arg (B30), Arg (B31) – NH_2 human insulin,

Arg (A0), His (A8), Asp (A18), Gly (A21), Asp (B3), Arg (B30), Lys (B31) – NH_2 human insulin.

In the pharmaceutical formulation as described above, the insulin analogue is alternatively selected from a group containing an insulin analogue of the formula II



where

A-1 is Lys, Arg or an amino group;

A0 is Lys, Arg or a chemical bond;

A1 is Arg or Gly;

A5 is Asp, Glu or Gln;

A15 is Asp, Glu or Gln;

A18 is Asp, Glu or Asn;

A21 is Ala, Ser, Thr or Gly;

B-1 is Asp, Glu or an amino group;

B0 is Asp, Glu or a chemical bond;

B1 is Asp, Glu, Phe or a chemical bond;

B3 is Asp, Glu or Asn;

B4 is Asp, Glu or Gln;

B29 is Arg, Lys or an amino acid selected from the group containing the amino acids Phe, Ala, Thr, Ser, Val, Leu, Glu or Asp, or a chemical bond;

B30 is Thr or a chemical bond;

B31 is Arg, Lys or a chemical bond;

B32 is Arg-amide or Lys-amide,

where not more than one amino acid residue from the group containing A5, A15, A18, B-1, B0, B1, B2, B3 and B4, simultaneously and independently of one another, is Asp or Glu, and the insulin analogue being selected in particular from a group containing:

Arg (A-1), Arg (A0), Glu (A5), His (A8), Gly (A21), Arg (B30) – NH_2 human insulin,

Arg (A-1), Arg (A0), Glu (A5), His (A8), Gly (A21), Lys (B30) – NH_2 human insulin,

Arg (A-1), Arg (A0), Glu (A15), His (A8), Gly (A21), Arg (B30) – NH_2 human insulin,

Arg (A-1), Arg (A0), Glu (A15), His (A8), Gly (A21), Lys (B30) – NH_2 human insulin,

Arg (A-1), Arg (A0), Asp (A18), His (A8), Gly (A21), Arg (B30) – NH_2 human insulin,

Arg (A-1), Arg (A0), Asp (A18), His (A8), Gly (A21), Arg (B30) – NH_2 human insulin,

Arg (A-1), Arg (A0), His (A8), Gly (A21), Glu (B0), Arg (B30) – NH_2 human insulin,

Arg (A-1), Arg (A0), His (A8), Gly (A21), Glu (B0), Lys (B30) – NH_2 human insulin,

Arg (A-1), Arg (A0), His (A8), Gly (A21), Asp (B3), Arg (B30) – NH_2 human insulin,

Arg (A-1), Arg (A0), His (A8), Gly (A21), Asp (B3), Lys (B30) – NH_2 human insulin,

Arg (A-1), Arg (A0), His (A8), Gly (A21), Glu (B4), Arg (B30) – NH_2 human insulin,

Arg (A-1), Arg (A0), His (A8), Gly (A21), Glu (B4), Lys (B30) – NH_2 human insulin,

Arg (A0), His (A8), Gly (A21), Arg (B31), Arg (B32) – NH_2 human insulin,

Arg (A0), His (A8), Gly (A21), Arg (B31), Lys (B32) – NH_2 human insulin,

Arg (A0), Glu (A5), His (A8), Gly (A21), Arg (B31), Arg (B32) – NH_2 human insulin,

Arg (A0), Glu (A5), His (A8), Gly (A21), Arg (B31), Lys (B32) – NH_2 human insulin,

Arg (A0), Asp (A18), His (A8), Gly (A21), Arg (B31), Arg (B32) – NH_2 human insulin,

Arg (A0), Asp (A18), His (A8), Gly (A21), Arg (B31), Lys (B32) – NH_2 human insulin,

Arg (A0), Glu (A15), His (A8), Gly (A21), Arg (B31), Arg (B32) – NH_2 human insulin,

Arg (A0), Glu (A15), His (A8), Gly (A21), Arg (B31), Lys (B32) – NH_2 human insulin,

Arg (A0), His (A8), Gly (A21), Asp (B3), Arg (B31), Arg (B32) – NH_2 human insulin,

Arg (A0), His (A8), Gly (A21), Asp (B3), Arg (B31), Lys (B32) – NH_2 human insulin,

Arg (A0), His (A8), Gly (A21), Glu (B4), Arg (B31), Arg (B32) – NH_2 human insulin,

Arg (A0), His (A8), Gly (A21), Glu (B4), Arg (B31), Lys (B32) – NH_2 human insulin,

Arg (A0), His (A8), Gly (A21), Glu (B0), Arg (B31), Arg (B32) – NH_2 human insulin,

Arg (A0), His (A8), Gly (A21), Glu (B0), Arg (B31), Lys (B32) – NH_2 human insulin,

Arg (A0), His (A8), Gly (A21), Arg (B30) – NH_2 human insulin,

Arg (A0), His (A8), Gly (A21), Lys (B30) – NH_2 human insulin,

Arg (A-1), Arg (A0), His (A8), Gly (A21), Arg (B30) – NH_2 human insulin,

Arg (A-1), Arg (A0), His (A8), Gly (A21), Lys (B30) – NH_2 human insulin,

Arg (A0), Arg (A1), His (A8), Gly (A21), Arg (B30) – NH_2 human insulin,

Arg (A0), Arg (A1), His (A8), Gly (A21), Lys (B30) – NH_2 human insulin,

His (A8), Gly (A21), Arg (B31), Arg (B32) – NH_2 human insulin.

In another alternative, in the pharmaceutical formulation as described above, the insulin derivative is selected from the group containing B29-N-myristoyl-des(B30) human insulin, B29-N-palmitoyl-des(B30) human insulin, B29-N-myristoyl human insulin, B29-N-palmitoyl human insulin, B28-N-myristoyl $Lys^{B28}Pro^{B29}$ human insulin, B28-N-palmitoyl-$Lys^{B28}Pro^{B29}$ human insulin, B30-N-myristoyl-$Thr^{B29}Lys^{B30}$ human insulin, B30-N-palmitoyl- $Thr^{B29}Lys^{B30}$ human insulin, B29-N-(N-palmitoyl-Y-glutamyl)-des(B39) human insulin, B29-N-(N-lithocholyl-Y-glutamyl)-des(B30) human insulin, B29-N-(ω-carboxyhepta-decanoyl)-des(B30) human insulin, and B29-N-(ω-carboxyheptadecanoyl) human insulin.

The invention further provides a pharmaceutical formulation as described above, comprising

0.001 to 0.2 mg/ml of zinc,

0.1 to 5.0 mg/ml of a preservative, and

5.0 to 100 mg/ml of an isotonicity agent.

The invention further provides a pharmaceutical formulation as described above, comprising a preservative selected from a group containing phenol, m-cresol, chlorocresol, benzyl alcohol, and parabens.

The invention further provides a pharmaceutical formulation as described above, comprising an isotonicity agent selected from a group containing mannitol, sorbitol, lactose, dextrose, trehalose, sodium chloride, and glycerol.

The invention further provides a pharmaceutical formulation as described above, having a pH in the range of pH 2.5 – 4.5, preferably pH 3.0 – 4.0, more preferably in the region of pH 3.75.

The invention further provides a pharmaceutical formulation as described above, the insulin, insulin analogue and/or insulin derivative being present in a concentration of 240 – 3000 nmol/ml.

The invention further provides a pharmaceutical formulation as described above, comprising glycerol at a concentration of 20 to 30 mg/ml.

The invention further provides a pharmaceutical formulation as described above, comprising glycerol at a concentration of 25 mg/ml.

The invention further provides a pharmaceutical formulation as described above, comprising m-cresol at a concentration of 1 to 3 mg/ml, preferably 2 mg/ml.

The invention further provides a pharmaceutical formulation as described above, comprising zinc at a concentration of 0.01 or 0.03 or 0.08 mg/ml.

The invention further provides a pharmaceutical formulation as described above, further comprising a glucagon-like peptide-1 (GLP1) or an analogue or derivative thereof, or exendin-3 and/or -4 or an analogue or derivative thereof, preferably exendin-4.

The invention further provides a pharmaceutical formulation as described above, in which an analogue of exendin-4 is selected from a group containing H-desPro^{36}-exendin-4-Lys$_6$-NH$_2$,

H-des($Pro^{36,37}$)-exendin-4-Lys_4-NH_2 and

H-des($Pro^{36,37}$)-exendin-4-Lys_5-NH_2,

or a pharmacologically tolerable salt thereof, or in which an analogue of exendin-4 is selected from the group containing

$desPro^{36}$ [Asp^{28}]exendin-4 (1-39),

$desPro^{36}$ [$IsoAsp^{28}$]exendin-4 (1-39),

$desPro^{36}$ [$Met(O)^{14}$, Asp^{28}]exendin-4 (1-39),

$desPro^{36}$ [$Met(O)^{14}$, $IsoAsp^{28}$]exendin-4 (1-39),

$desPro^{36}$ [$Trp(O_2)^{25}$, Asp^{28}]exendin-2 (1-39),

$desPro^{36}$ [$Trp(O_2)^{25}$, $IsoAsp^{28}$]exendin-2 (1-39),

$desPro^{36}$ [$Met(O)^{14}Trp(O_2)^{25}$, Asp^{28}]exendin-4 (1-39) and

$desPro^{36}$ [$Met(O)^{14}Trp(O_2)^{25}$, $IsoAsp^{28}$]exendin-4 (1-39),

or a pharmacologically tolerable salt thereof.

The invention further provides a pharmaceutical formulation as described above in which the peptide Lys_6-NH_2 is attached to the C-termini of the analogues of exendin-4.

The invention further provides a pharmaceutical formulation as described above, in which an analogue of exendin-4 is selected from the group containing

H-$(Lys)_6$- des Pro^{36} [Asp^{28}]exendin-4(1-39)-Lys_6-NH_2

des $Asp^{28}Pro^{36}$, Pro^{37}, Pro^{38} exendin-4(1-39) -NH_2,

H-$(Lys)_6$- des Pro^{36}, Pro^{37}, Pro^{38} [Asp^{28}]exendin-4(1-39) -NH_2,

H-Asn-$(Glu)_5$ des Pro^{36}, Pro^{37}, Pro^{38} [Asp^{28}]exendin-4(1-39) -NH_2,

des Pro^{36}, Pro^{37}, Pro^{38} [Asp^{28}]exendin-4(1-39)-$(Lys)_6$-NH_2,

H-$(Lys)_6$- des Pro^{36}, Pro^{37}, Pro^{38} [Asp^{28}]exendin-4(1-39)-$(Lys)_6$-NH_2,

H-Asn-$(Glu)_5$- des Pro^{36}, Pro^{37}, Pro^{38} [Asp^{28}]exendin-4(1-39)-$(Lys)_6$-NH_2,

H-$(Lys)_6$- des Pro^{36} [$Trp(O_2)^{25}$, Asp^{28}]exendin-4(1-39)-Lys_6-NH_2,

H- des Asp^{28} Pro^{36}, Pro^{37}, Pro^{38} [$Trp(O_2)^{25}$]exendin-4(1-39) -NH_2,

H-$(Lys)_6$- des Pro^{36}, Pro^{37}, Pro^{38} [$Trp(O_2)^{25}$, Asp^{28}]exendin-4(1-39) -NH_2,

H-Asn-$(Glu)_5$- des Pro^{36}, Pro^{37}, Pro^{38} [$Trp(O_2)^{25}$, Asp^{28}]exendin-4(1-39) -NH_2,

des Pro^{36}, Pro^{37}, Pro^{38} [$Trp(O_2)^{25}$, Asp^{28}]exendin-4(1-39)-$(Lys)_6$-NH_2,

H-$(Lys)_6$- des Pro^{36}, Pro^{37}, Pro^{38} [$Trp(O_2)^{25}$, Asp^{28}]exendin-4(1-39)-$(Lys)_6$-NH_2,
H-Asn-$(Glu)_5$- des Pro^{36}, Pro^{37}, Pro^{38} [$Trp(O_2)^{25}$, Asp^{28}]exendin-4(1-39)-$(Lys)_6$-NH_2,
H-$(Lys)_6$- des Pro^{36} [$Met(O)^{14}$, Asp^{28}]exendin-4(1-39)-Lys_6-NH_2,
des $Met(O)^{14}$ Asp^{28} Pro^{36}, Pro^{37}, Pro^{38} exendin-4(1-39) -NH_2,
H-$(Lys)_6$- des Pro^{36}, Pro^{37}, Pro^{38} [$Met(O)^{14}$, Asp^{28}]exendin-4(1-39) -NH_2,
H-Asn-$(Glu)_5$- des Pro^{36}, Pro^{37}, Pro^{38} [$Met(O)^{14}$, Asp^{28}] exendin-4(1-39) -NH_2,
des Pro^{36}, Pro^{37}, Pro^{38} [$Met(O)^{14}$, Asp^{28}]exendin-4(1-39)-$(Lys)_6$-NH_2,
H-$(Lys)_6$- des Pro^{36}, Pro^{37}, Pro^{38} [$Met(O)^{14}$, Asp^{28}]exendin-4(1-39)-Lys_6-NH_2,
H-Asn-$(Glu)_5$ des Pro^{36}, Pro^{37}, Pro^{38} [$Met(O)^{14}$, Asp^{28}] exendin-4(1-39)-$(Lys)_6$-NH_2,
H-$(Lys)_6$- des Pro^{36} [$Met(O)^{14}$, $Trp(O_2)^{25}$, Asp^{28}]exendin-4(1-39)-Lys_6-NH_2,
des Asp^{28} Pro^{36}, Pro^{37}, Pro^{38} [$Met(O)^{14}$, $Trp(O_2)^{25}$]exendin-4(1-39) -NH_2,
H-$(Lys)_6$- des Pro^{36}, Pro^{37}, Pro^{38} [$Met(O)^{14}$, $Trp(O_2)^{25}$, Asp^{28}]exendin-4(1-39) -NH_2,
H-Asn-$(Glu)_5$- des Pro^{36}, Pro^{37}, Pro^{38} [$Met(O)^{14}$, Asp^{28}] exendin-4(1-39) -NH_2,
des Pro^{36}, Pro^{37}, Pro^{38} [$Met(O)^{14}$, $Trp(O_2)^{25}$, Asp^{28}]exendin-4(1-39)-$(Lys)_6$-NH_2,
H-$(Lys)_6$- des Pro^{36}, Pro^{37}, Pro^{38} [$Met(O)^{14}$, $Trp(O_2)^{25}$, Asp^{28}]exendin-4(1-39)-$(Lys)_6$-NH_2,
H-Asn-$(Glu)_5$- des Pro^{36}, Pro^{37}, Pro^{38} [$Met(O)^{14}$, $Trp(O_2)^{25}$, Asp^{28}] exendin-4(1-39)-$(Lys)_6$-NH_2,
or a pharmacologically tolerable salt thereof.

The invention further provides a pharmaceutical formulation as described above, further comprising Arg^{34}, Lys^{26} (N^{ε}(γ-glutamyl(N^{α}-hexadecanoyl))) GLP-1 (7-37) [liraglutide] or a pharmacologically tolerable salt thereof.

The invention further provides a pharmaceutical formulation as described above, comprising methionine in a concentration range of up to 10 mg/ml, preferably up to 3 mg/ml.

The invention further provides a process for preparing a formulation as described above, which comprises

(a) introducing the components into an aqueous solution and

(b) adjusting the pH.

The invention further provides for the use of a formulation as described above for treating diabetes mellitus.

The invention provides a medicament for treating diabetes mellitus, composed of a formulation as described above.

The specification is described below with reference to a number of examples, which are not intended to have any restrictive effect whatsoever.

Key to figures:

Fig. 1: blood sugar reducing effect of new insulin analogues of formula I in rats

Fig. 2: blood sugar reducing effect of new insulin analogues of formula I in dogs

Fig. 3: blood sugar reducing effect of YKL205 in dogs

Fig. 4: zinc dependence of hypoglycemic effect of YKL205 in dogs

Fig. 5: blood sugar reducing effect of inventive insulin analogues of formula II in rats

Fig. 6: blood sugar reducing effect of insulin glargine in rats

Examples:

The examples below are intended to illustrate the concept of the invention, without having any restricting effect.

Example 1: Studies on the dispensing of the solution using nitrogen, oxygen, and dispensing under standard conditions

The solution is prepared by introducing about 25% of 0.1 M HCl and adding 0.2% of Polysorbate 20 stock solution. In succession, SAR161271 and the zinc chloride stock solution are added and stirred. Adding 1 M HCl at a pH of pH 2 dissolves SAR161271. The solution is stirred and then 1 M NaOH is added to adjust the pH to pH 4.0. Injection-grade water is used to make up to 90% of the batch size. Added to this solution in succession with stirring are glycerol 85% and m-cresol. Injection-grade water is used to make up to the desired final weight. The solution is filtered using a filter attachment on a syringe. The batch was divided into three: ungassed (as reference), gassed with nitrogen and gassed with oxygen (as a positive control). Gassing took place by blanketing with the gas in question.

Untreated

Amount of SAR161271

1 M + 5°C: 3.67 mg/ml

1 M + 25°C: 3.46 mg/ml

1 M + 37°C: 3.41 mg/ml

Impurities

1 M + 5°C: 3.0%

1 M + 25°C: 3.6%

1 M + 37°C: 5.6%

High molecular mass proteins

1 M + 5°C: 0.2%

1 M + 25°C: 0.3%

1 M + 37°C:1.4%

Nitrogen treated

Amount of SAR161271

1 M + 5°C: 3.73 mg/ml

1 M + 25°C: 3.50 mg/ml

1 M + 37°C: 3.35 mg/ml

Impurities

1 M + 5°C: 3.1%

1 M + 25°C: 3.5%

1 M + 37°C: 5.2%

High molecular mass proteins

1 M + 5°C: 0.2%

1 M + 25°C: 0.3%

1 M + 37°C:1.2%

Oxygen treated

Amount of SAR161271

1 M + 5°C: 3.54 mg/ml

1 M + 25°C: 3.34 mg/ml

1 M + 37°C: 3.26 mg/ml

Impurities

1 M + 5°C: 3.2%

1 M + 25°C: 3.9%

1 M + 37°C: 7.2%

High molecular mass proteins

1 M + 5°C: 0.2%

1 M + 25°C: 0.5%

1 M + 37°C:2.9%

In the case of dispensing using nitrogen, there was no distinct reduction in impurities after 1 month as compared with the untreated sample. In the case of dispensing using oxygen, slightly higher impurities and high molecular mass proteins were apparent. On the basis of these results, dispensing under standard conditions was selected.

Example 2: Study of stability with 3 different antioxidants

The solution was prepared as described in example 1. In addition, between the addition of glycerol 85% and m-cresol, the antioxidants - methionine or glutathione or ascorbic acid - were added to the formulation in order to reduce the level of oxidative by-product. The formulations containing either glutathione

(0.183 mg/ml) or ascorbic acid (0.105 mg/ml) showed a distinct discoloration after just 3 months of storage. The formulation containing methionine (0.089 mg/ml) showed no discoloration at all and was stable after 1 month of storage at 5°C.

Amount of SAR161271

1 M + 5°C: 3.43 mg/ml

1 M + 25°C: 3.43 mg/ml

1 M + 37°C: 3.53 mg/ml

Impurities

1 M + 5°C: 2.9%

1 M + 25°C: 3.4%

1 M + 37°C: 5.7%

High molecular mass proteins

1 M + 5°C: 0.2%

1 M + 25°C: 0.3%

1 M + 37°C: 1.1%

Example 3: Formulation of amidated insulin derivatives

Examples 3 to 7 serve only for the determination of the biological, pharmacological, and physicochemical properties of insulin analogues of formula I, involving first the provision of formulations thereof (example 3) and then the conduct of corresponding tests (examples 4 to 7). A solution with the compounds was prepared as follows: the insulin analogue of the invention was dissolved with a target concentration of 240 ± 5 µM in 1 mM hydrochloric acid with 80 µg/ml zinc (as zinc chloride).

The compositions used as dissolution medium were as follows:

a) 1 mM hydrochloric acid

b) 1 mM hydrochloric acid, 5 µg/ml zinc (added as zinc chloride or hydrochloric acid)

c) 1 mM hydrochloric acid, 10 µg/ml zinc (added as zinc chloride or hydrochloric acid)

d) 1 mM hydrochloric acid, 15 µg/ml zinc (added as zinc chloride or hydrochloric acid)

e) 1 mM hydrochloric acid, 30 µg/ml zinc (added as zinc chloride or hydrochloric acid)

f) 1 mM hydrochloric acid, 80 µg/ml zinc (added as zinc chloride or hydrochloric acid)

g) 1 mM hydrochloric acid, 120 µg/ml zinc (added as zinc chloride or hydrochloric acid)

For this purpose, an amount of the freeze-dried material higher by around 30% than the amount needed on the basis of the molecular weight and the target concentration was first weighed out. Thereafter the existing concentration was determined by means of analytical HPLC and the solution was then made up with 5 mM hydrochloric acid with 80 µg/ml zinc to the volume needed in order to achieve the target concentration. If necessary, the pH was readjusted to 3.5 ± 0.1. Following final analysis by HPLC to ensure the target concentration of 240 ± 5 µM, the completed solution was transferred, using a syringe having a 0.2 µm filter attachment, into a sterile vial which was closed with a septum and a crimped cap. For the short-term, single testing of the insulin derivatives of the invention, there was no optimization of the formulations, in relation, for example, to addition of isotonic agents, preservatives or buffer substances.

Example 4: Evaluation of the blood sugar-reducing action of new insulin analogues in rats

The blood sugar-lowering effect of selected new insulin analogues is tested in healthy male normoglycemic Wistar rats. Male rats receive a subcutaneous injection of a dose of 9 nmol/kg of an insulin analogue. Immediately before the injection of the insulin analogue and at regular intervals for up to eight hours after injection, blood samples are taken from the animals, and their blood sugar

content determined. The experiment shows clearly (cf. fig. 1) that the insulin analogue of the invention leads to a significantly retarded onset of action and to a longer, uniform duration of action.

Example 5: Evaluation of the blood sugar-reducing action of new insulin analogues in dogs

The blood sugar-lowering effect of selected new insulin analogues is tested in healthy male normoglycemic beagles. Male animals receive a subcutaneous injection of a dose of 6 nmol/kg of an insulin analogue. Immediately before the injection of the insulin analogue and at regular intervals for up to forty-eight hours after injection, blood samples are taken from the animals, and their blood sugar content determined. The experiment shows clearly (cf. fig. 2) that the insulin analogue of the invention that is used leads to a significantly retarded onset of action and to a longer, uniform duration of action.

Example 6: Evaluation of the blood sugar-reducing action in dogs with twofold-increased dose

The blood sugar-lowering effect of selected new insulin analogues is tested in healthy male normoglycemic beagles. Male animals receive a subcutaneous injection of a dose of 6 nmol/kg and 12 nmol/kg of an insulin analogue. Immediately before the injection of the insulin analogue and at regular intervals for up to forty-eight hours after injection, blood samples are taken from the animals, and their blood sugar content determined. The experiment shows clearly (cf. fig. 3) that the insulin analogue of the invention that is used has a dose-dependent effect, but that, despite the twofold-increased dose, the effect profile is flat, i.e., there is no pronounced low point (nadir) observed. From this it may be inferred that the insulins of the invention, in comparison to known retarded insulins, lead to significantly fewer hypoglycemic events.

Example 7: Evaluation of the blood sugar-reducing effect in dogs with different concentrations of zinc in the formulation

The experiments were carried out as described in example 5. Figure 4 shows the result. Accordingly, the time/activity curve of the insulin analogue of the invention can be influenced through the amount of zinc ions in the formulation, with the same concentration of insulin, in such a way that a rapid onset of action is observed at zero or low zinc content and the action persists over 24 hours, whereas, with a higher zinc content, a flat onset of action is observed and the insulin effect persists for much longer than 24 hours.

Example 8: Formulation of amidated insulin derivatives

Examples 8 to 10 serve only for the determination of the biological, pharmacological, and physicochemical properties of insulin analogues of formula II, involving first the provision of formulations thereof (example 8) and then the conduct of corresponding tests (examples 9 and 10). The insulin analogue of the invention was dissolved with a target concentration of 240 ± 5 µM in 1 mM hydrochloric acid with 80 µg/ml zinc (as zinc chloride). For this purpose, an amount of the freeze-dried material higher by around 30% than the amount needed on the basis of the molecular weight and the target concentration was first weighed out. Thereafter the existing concentration was determined by means of analytical HPLC and the solution was then made up with 5 mM hydrochloric acid with 80 µg/ml zinc to the volume needed in order to achieve the target concentration. If necessary, the pH was readjusted to 3.5 ± 0.1. Following final analysis by HPLC to ensure the target concentration of 240 ± 5 µM, the completed solution was transferred, using a syringe having a 0.2 µm filter attachment, into a sterile vial which was closed with a septum and a crimped cap. For the short-term, single testing of the insulin derivatives of the invention, there was no optimization of the formulations, in relation, for example, to addition of isotonic agents, preservatives or buffer substances.

Example 9: Evaluation of the blood sugar-reducing action of new insulin analogues in rats

The blood sugar-lowering effect of selected new insulin analogues is tested in healthy male normoglycemic Wistar rats. Male rats receive a subcutaneous injection of a dose of 9 nmol/kg of an insulin analogue. Immediately before the injection of the insulin analogue and at regular intervals for up to eight hours after injection, blood samples are taken from the animals, and their blood sugar content determined. The experiment shows clearly (cf. fig. 5) that the insulin analogue of the invention leads to a significantly retarded onset of action and to a longer, uniform duration of action.

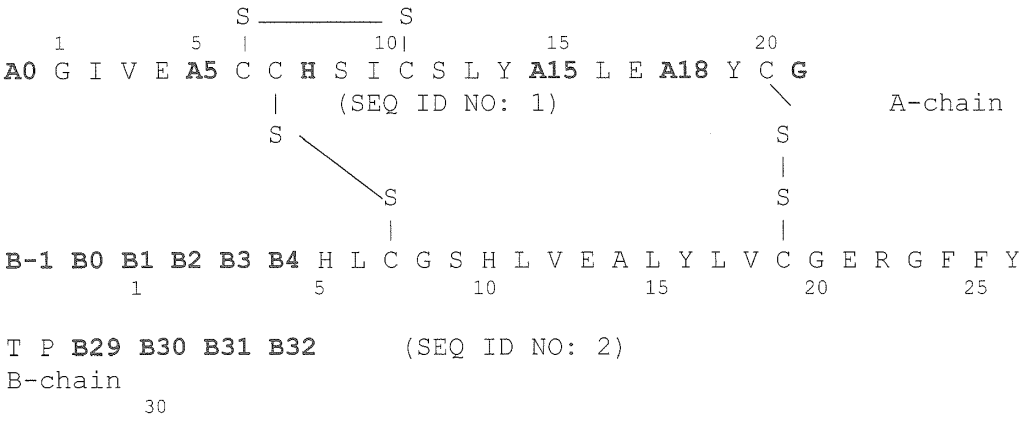
Example 10: Evaluation of the blood sugar-reducing action of new insulin analogues in dogs

The blood sugar-lowering effect of selected new insulin analogues is tested in healthy male normoglycemic beagles. Male animals receive a subcutaneous injection of a dose of 6 nmol/kg of an insulin analogue. Immediately before the injection of the insulin analogue and at regular intervals for up to forty-eight hours after injection, blood samples are taken from the animals, and their blood sugar content determined. The experiment shows clearly that the insulin analogue of the invention leads to a significantly retarded, flat onset of action and to a longer, uniform duration of action.

Patentkrav

1. Vandig farmasøytisk formulering omfattende en insulinanalog eller et insulinderivat, eller et farmakologisk tolererbart salt derav, og metionin, hvor pH-verdien av formuleringen er 4,5 eller mindre, og hvor

(I) insulinanalogen er utvalgt fra en gruppe bestående av Gly(A21), Arg(B31), Arg(B32)-humant insulin, Lys(B3), Glu(B29)-humant insulin, Asp(B28)-humant insulin, Lys(B28) Pro(B29)-humant insulin, -des(B30)-humant insulin og en insulinanalog med formel I



hvor

A0 tilsvarer Lys eller Arg;
A5 tilsvarer Asp, Gln eller Glu;
A15 tilsvarer Asp, Glu eller Gln;
A18 tilsvarer Asp, Glu eller Asn;
B-1 tilsvarer Asp, Glu eller en aminogruppe;
B0 tilsvarer Asp, Glu eller en kjemisk binding;
B1 tilsvarer Asp, Glu eller Phe;
B2 tilsvarer Asp, Glu eller Val;
B3 tilsvarer Asp, Glu eller Asn;
B4 tilsvarer Asp, Glu eller Gln;
B29 tilsvarer Lys eller en kjemisk binding;
B30 tilsvarer Thr eller en kjemisk binding;
B31 tilsvarer Arg, Lys eller en kjemisk binding;
B32 tilsvarer Arg-amid, Lys-amid eller en aminogruppe,

hvor to aminosyrerester av gruppen bestående av A5, A15, A18, B-1, B0, B1, B2, B3 og B4 samtidig og uavhengig av hverandre tilsvarer Asp eller Glu, og hvor insulinanalogen fortrinnsvis er utvalgt fra en gruppe bestående av:

Arg (A0), His (A8), Glu (A5), Asp (A18), Gly (A21), Arg (B31), Arg (B32) - NH_2 humant insulin,

Arg (A0), His (A8), Glu (A5), Asp (A18), Gly (A21), Arg (B31), Lys (B32) - NH_2 humant insulin,

Arg (A0), His (A8), Glu (A15), Asp (A18), Gly (A21), Arg (B31), Arg (B32) - NH_2 humant insulin,

Arg (A0), His (A8), Glu (A15), Asp (A18), Gly (A21), Arg (B31), Lys (B32) - NH_2 humant insulin,

Arg (A0), His (A8), Glu (A5), Glu (A15), Gly (A21), Arg (B31), Arg (B32)- NH_2 humant insulin,

Arg (A0), His (A8), Glu (A5), Glu (A15), Gly (A21), Arg (B31), Lys (B32)- NH_2 humant insulin,

Arg (A0), His(A8), Glu (A5), Gly (A21), Asp (B3), Arg (B31), Arg (B32)- NH_2 humant insulin,

Arg (A0), His(A8), Glu (A5), Gly (A21), Asp (B3), Arg (B31), Lys (B32)- NH_2 humant insulin,

Arg (A0), His (A8), Glu (A15), Gly (A21), Asp (B3), Arg (B31), Arg (B32)- NH_2 humant insulin,

Arg (A0), His (A8), Glu (A15), Gly (A21), Asp (B3), Arg (B31), Lys (B32)- NH_2 humant insulin,

Arg (A0), His (A8), Asp (A18), Gly (A21), Asp (B3), Arg (B31), Arg (B32)- NH_2 humant insulin,

Arg (A0), His (A8), Asp (A18), Gly (A21), Asp (B3), Arg (B31), Lys (B32)- NH_2 humant insulin,

Arg (A0), His (A8), Gly (A21), Asp (B3), Glu (B4), Arg (B31), Arg (B32)- NH_2 humant insulin,

Arg (A0), His (A8), Gly (A21), Asp (B3), Glu (B4), Arg (B31), Lys (B32) - NH_2 humant insulin,

Arg (A0), His (A8), Glu (A5), Gly (A21), Glu (B4), Arg (B31), Arg (B32)- NH_2 humant insulin,

Arg (A0), His (A8), Glu (A5), Gly (A21), Glu (B4), Arg (B31), Lys (B32)- NH_2 humant insulin,

Arg (A0), His (A8), Glu (A15), Gly (A21), Glu (B4), Arg (B31), Arg (B32) - NH_2 humant insulin,

Arg (A0), His (A8), Glu (A15), Gly (A21), Glu (B4), Arg (B31), Lys (B32) - NH_2 humant insulin,

Arg (A0), His (A8), Asp (A18), Gly (A21), Glu (B4), Arg (B31), Arg (B32)- NH_2 humant insulin,

Arg (A0), His (A8), Asp (A18), Gly (A21), Glu (B4), Arg (B31), Lys (B32)- NH_2 humant insulin,

Arg (A0), His (A8), Glu (A5), Gly (A21), Glu (B0), Arg (B31), Arg (B32)- NH_2 humant insulin,

Arg (A0), His (A8), Glu (A5), Gly (A21), Glu (B0), Arg (B31), Lys (B32)- NH_2 humant insulin,

Arg (A0), His (A8), Glu (A15), Gly (A21), Glu (B0), Arg (B31), Arg (B32)- NH_2 humant insulin,

Arg (A0), His (A8), Glu (A15), Gly (A21), Glu (B0), Arg (B31), Lys (B32) - NH_2 humant insulin,

Arg (A0), His (A8), Asp (A18), Gly (A21), Glu (B0), Arg (B31), Arg (B32) - NH_2 humant insulin,

Arg (A0), His (A8), Asp (A18), Gly (A21), Glu (B0), Arg (B31), Lys (B32) - NH_2 humant insulin,

Arg (A0), His (A8), Glu (A5), Gly (A21), Asp (B1), Arg (B31), Arg (B32)- NH_2 humant insulin,

Arg (A0), His (A8), Glu (A5), Gly (A21), Asp (B1), Arg (B31), Lys (B32)- NH_2 humant insulin,

Arg (A0), His (A8), Glu (A15), Gly (A21), Asp (B1), Arg (B31), Arg(B32) - NH_2 humant insulin,

Arg (A0), His (A8), Glu (A15), Gly (A21), Asp (B1), Arg (B31), Lys (B32) - NH_2 humant insulin,

Arg (A0), His (A8), Asp (A18), Gly (A21), Asp (B1), Arg (B31), Arg (B32)- NH_2 humant insulin,

Arg (A0), His (A8), Asp (A18), Gly (A21), Asp (B1), Arg (B31), Lys (B32)- NH_2 humant insulin,

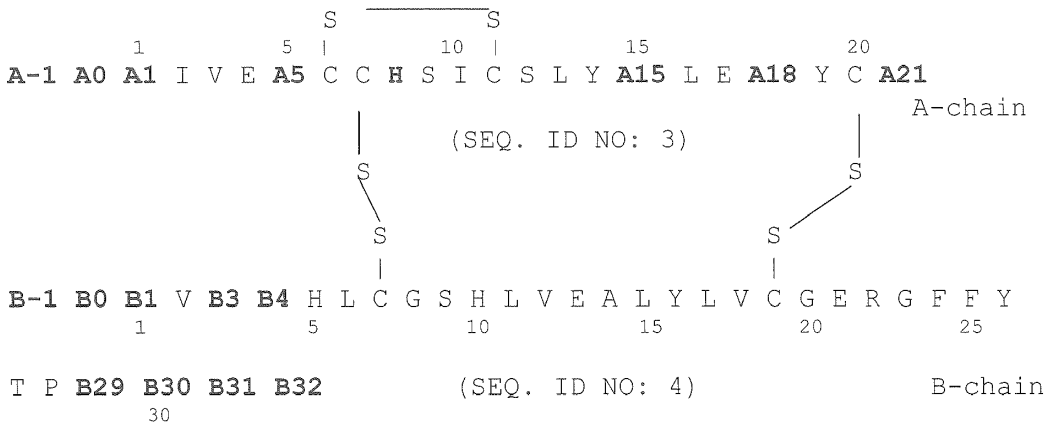
Arg (A0), His (A8), Gly (A21), Glu (B0), Asp (B1), Arg (B31), Arg (B32) - NH_2 humant insulin,

Arg (A0), His (A8), Gly (A21), Glu (B0), Asp (B1), Arg (B31), Lys (B32) - NH_2 humant insulin,

Arg (A0), His (A8), Asp (A18), Gly (A21), Asp (B3), Arg (B30), Arg (B31)- NH_2 humant insulin,

Arg (A0), His (A8), Asp (A18), Gly (A21), Asp (B3), Arg (B30), Lys (B31)- NH_2 humant insulin,

(II) insulinanalogen er utvalgt fra en gruppe bestående av en insulinanalog med formel II



hvor

A-1 tilsvarer Lys, Arg eller en aminogruppe;
A0 tilsvarer Lys, Arg eller en kjemisk binding;
A1 tilsvarer Arg eller Gly;
A5 tilsvarer Asp, Glu eller Gln;
A15 tilsvarer Asp, Glu eller Gln;
A18 tilsvarer Asp, Glu eller Asn;
A21 tilsvarer Ala, Ser, Thr eller Gly;
B-1 tilsvarer Asp, Glu eller en aminogruppe;
B0 tilsvarer Asp, Glu eller en kjemisk binding;
B1 tilsvarer Asp, Glu, Phe eller en kjemisk binding;
B3 tilsvarer Asp, Glu eller Asn;
B4 tilsvarer Asp, Glu eller Gln;

B29 tilsvarer Arg, Lys eller en aminosyre utvalgt fra en gruppe bestående av aminosyrene Phe, Ala, Thr, Ser, Val, Leu, Glu eller Asp, eller en kjemisk binding;
B30 tilsvarer Thr eller en kjemisk binding;
B31 tilsvarer Arg, Lys eller en kjemisk binding;
B32 tilsvarer Arg-amid eller Lys-amid

hvor ikke mer enn én aminosyrerest fra gruppen bestående av A5, A15, A18, B-1, B0, B1, B2, B3 og B4 samtidig og uavhengig av hverandre tilsvarer Asp eller Glu,
og hvor insulinanalogen fortrinnsvis er utvalgt fra en gruppe bestående av:

Arg (A-1), Arg (A0), Glu (A5), His (A8), Gly (A21), Arg (B30) - NH_2 humant insulin,

Arg (A-1), Arg (A0), Glu (A5), His (A8), Gly (A21), Lys (B30) - NH_2 humant insulin,
Arg (A-1), Arg (A0), Glu (A15), His (A8), Gly (A21), Arg (B30) - NH_2 humant insulin,
Arg (A-1), Arg (A0), Glu (A15), His (A8), Gly (A21), Lys (B30) - NH_2 humant insulin,
Arg (A-1), Arg (A0), Asp (A18), His (A8), Gly (A21), Arg (B30) - NH_2 humant insulin,
Arg (A-1), Arg (A0), His (A8), Gly (A21), Glu (B0), Arg (B30) - NH_2 humant insulin,
Arg (A-1), Arg (A0), His (A8), Gly (A21), Glu (B0), Lys (B30) - NH_2 humant insulin,
Arg (A-1), Arg (A0), His (A8), Gly (A21), Asp (B3), Arg (B30) - NH_2 humant insulin,
Arg (A-1), Arg (A0), His (A8), Gly (A21), Asp (B3), Lys (B30) - NH_2 humant insulin,
Arg (A-1), Arg (A0), His (A8), Gly (A21), Glu (B4), Arg (B30) - NH_2 humant insulin,
Arg (A-1), Arg (A0), His (A8), Gly (A21), Glu (B4), Lys (B30) - NH_2 humant insulin,
Arg (A0), His (A8), Gly (A21), Arg (B31), Arg (B32) - NH_2 - humant insulin,
Arg (AO), His (A8), Gly (A21), Arg (B31), Lys (B32) - NH_2 - humant insulin,
Arg (A0), Glu (A5), His (A8), Gly (A21), Arg (B31), Arg (B32) - NH_2 - humant insulin,
Arg (A0), Glu (A5), His (A8), Gly (A21), Arg (B31), Lys (B32) - NH_2 - humant insulin,
Arg (A0), Asp (A18), His (A8), Gly (A21), Arg (B31), Arg (B32) - NH_2 - humant insulin,
Arg (A0), Asp (A18), His (A8), Gly (A21), Arg (B31), Lys (B32) - NH_2- humant insulin,
Arg (A0), Glu (A15), His (A8), Gly (A21), Arg (B31), Arg (B32) - NH_2 - humant insulin,
Arg (A0), Glu (A15), His (A8), Gly (A21), Arg (B31), Lys (B32) - NH_2 - humant insulin,
Arg (A0), His (A8), Gly (A21), Asp (B3), Arg (B31), Arg (B32) - NH_2 - humant insulin,
Arg (A0), His (A8), Gly (A21), Asp (B3), Arg (B31), Lys (B32) - NH_2 - humant insulin,
Arg (A0), His (A8), Gly (A21), Glu (B4), Arg (B31), Arg (B32) - NH_2 - humant insulin,
Arg (A0), His (A8), Gly (A21), Glu (B4), Arg (B31), Lys (B32) - NH_2 - humant insulin,

Arg (A0), His (A8), Gly (A21), Glu (B0), Arg (B31), Arg (B32) - NH_2 - humant insulin,
Arg (A0), His (A8), Gly (A21), Glu (B0), Arg (B31), Lys (B32) - NH_2 - humant insulin,
Arg (A0), His (A8), Gly (A21), Arg (B30) - NH_2 - humant insulin,
Arg (A0), His (A8), Gly (A21), Lys (B30) - NH_2 - humant insulin,
Arg (A-1), Arg (A0), His (A8), Gly (A21), Arg (B30) - NH_2 - humant insulin,
Arg (A-1), Arg (A0), His (A8), Gly (A21), Lys (B30) - NH_2 - humant insulin,
Arg (A0), Arg (A1), His (A8), Gly (A21), Arg (B30) - NH_2 - humant insulin,
Arg (A0), Arg (A1), His (A8), Gly (A21), Lys (B30) - NH_2 - humant insulin,
His (A8), Gly (A21), Arg (B31), Arg (B32) - NH_2 - humant insulin, eller

(III) insulinderivatet er utvalgt fra en gruppe bestående av B29-N-myristoyl-des(B30) humant insulin, B29-N-palmitoyl-des(B30) humant insulin, B29-N-myristoyl humant insulin, B29-N-palmitoyl humant insulin, B28-N-myristoyl $Lys^{B28}Pro^{B29}$ humant insulin, B28-N-palmitoyl-$Lys^{B28}Pro^{B29}$ humant insulin, B30-N-myristoyl-$Thr^{B29}Lys^{B30}$ humant insulin, B30-N-palmitoyl-$Thr^{B29}Lys^{B30}$ humant insulin, B29-N-(N-palmitoyl-Y-glutamyl)-des(B39) humant insulin, B29-N-(N-litokolyl-Y-glutamyl)-des(B30) humant insulin, B29-N-(ω-karboksyhepta-dekanoyl)-des(B30) humant insulin og B29-N-(ω-karboksyheptadekanoyl) humant insulin.

2. Farmasøytisk formulering ifølge krav 1, som omfatter
0,001 til 0,2 mg/ml sink,
0,1 til 5,0 mg/ml av et konserveringsmiddel og
5,0 til 100 mg/ml av et isotonisitetsmiddel

3. Farmasøytisk formulering ifølge krav 1 eller 2, omfattende et konserveringsmiddel utvalgt fra en gruppe bestående av fenol, m-kresol, klorkresol, benzylalkohol, paraben og/eller et isotonisitetsmiddel utvalgt fra en gruppe bestående av mannitol, sorbitol, lactose, dekstrose, trehalose, natriumklorid og glyserol.

4. Farmasøytisk formulering ifølge ett eller flere av kravene 1 til 3, med en pH-verdi i området fra pH 2,5 - 4,5, fortrinnsvis i området fra pH 3,0 - 4,0, og spesielt foretrukket i området fra pH 3,75.

5. Farmasøytisk formulering ifølge ett eller flere av kravene 1 til 4, hvor insulinanalogen og/eller insulinderivatet foreligger i en konsentrasjon på 240 - 3000 nmol/ml.

6. Farmasøytisk formulering ifølge ett eller flere av kravene 1 til 5, omfattende glyserol i en konsentrasjon på 20 til 30 mg/ml, fortrinnsvis i en konsentrasjon på 25 mg/ml.

7. Farmasøytisk formulering ifølge ett eller flere av kravene 1 til 6, omfattende m-kresol i en konsentrasjon på 1 til 3 mg/ml, fortrinnsvis i en konsentrasjon på 2 mg/ml.

8. Farmasøytisk formulering ifølge ett eller flere av kravene 1 til 7, omfattende sink i en konsentrasjon på 0,01 eller 0,03 eller 0,08 mg/ml.

9. Farmasøytisk formulering ifølge ett eller flere av kravene 1 til 8, som ytterligere omfatter et glukagon-liknende peptid-1 (GLP1) eller en analog eller et derivat derav, eller exendin-3 eller -4 eller en analog eller et derivat derav.

10. Farmasøytisk formulering ifølge krav 9, som omfatter:

(i) exendin-4,
(ii) en analog av exendin-4 utvalgt fra en gruppe bestående av
H-desPro^{36}-exendin-4-Lys$_6$-NH$_2$,
H-des(Pro^{36,37})-exendin-4-Lys$_4$-NH$_2$ og
H-des(Pro^{36,37})-exendin-4-Lys$_5$-NH$_2$,
eller et farmakologisk tolererbart salt derav, eller
(iii) en analog av exendin-4 utvalgt fra en gruppe bestående av
desPro^{36} [Asp^{28}] exendin-4 (1-39),
desPro^{36} [IsoAsp^{28}] exendin-4 (1-39),
desPro^{36} [Met(O)^{14}, Asp^{28}] exendin-4 (1-39),
desPro^{36} [Met(O)^{14}, IsoAsp^{28}] exendin-4 (1-39),
desPro^{36} [Trp(O$_2$)^{25}, Asp^{28}] exendin-2 (1-39),
desPro^{36} [Trp(O$_2$)^{25}, IsoAsp^{28}] exendin-2 (1-39),
desPro^{36} [Met(O)^{14}Trp(O$_2$)^{25}, Asp^{28}] exendin-4 (1-39) og
desPro^{36} [Met(O)^{14}Trp(O$_2$)^{25}, IsoAsp^{28}] exendin-4 (1-39),
eller et farmakologisk tolererbart salt derav,
hvor eventuelt på C-Termini av analogene av exendin-4 er peptidet -Lys$_6$-NH2 tilføyd.

11. Farmasøytisk formulering ifølge krav 9, i hvilken analogen av exendin-4 utvelges fra en gruppe bestående av
H-(Lys)$_6$-des Pro^{36} [Asp^{28}]Exendin-4(1-39)-Lys$_6$-NH$_2$
des Asp^{28}Pro^{36}, Pro^{37}, Pro$_{38}$ Exendin-4(1-39)-NH$_2$,
H-(Lys)$_6$-des Pro^{36}, Pro^{37}, Pro^{38} [Asp^{28}]Exendin-4(1-39)-NH$_2$,

H-Asn-$(Glu)_5$-des Pro^{36}, Pro^{37}, Pro^{38} [Asp^{28}]Exendin-4(1-39)-NH_2,
des Pro^{36}, Pro^{37}, Pro^{38} [Asp^{28}]Exendin-4(1-39)-$(Lys)_6$-NH_2,
H-$(Lys)_6$-des Pro^{36}, Pro^{37}, Pro^{38} [Asp^{28}]Exendin-4(1-39)-$(Lys)_6$-NH_2,
H-Asn-$(Glu)_5$-des Pro^{36}, Pro^{37}, Pro^{38} [Asp^{28}]Exendin-4(1-39)-$(Lys)_6$-NH_2,
H-$(Lys)_6$-des Pro^{36} [$Trp(O_2)^{25}$, Asp^{28}]Exendin-4(1-39)-Lys_6-NH_2,
H-desAsp^{28} Pro^{36}, Pro^{37}, Pro^{38} [$Trp(O_2)^{25}$]Exendin-4(1-39-NH_2,
H-$(Lys)_6$-des Pro^{36}, Pro^{37}, Pro^{38} [$Trp(O_2)^{25}$, Asp^{28}]Exendin-4(1-39) -NH_2,
H-Asn-$(Glu)_5$-des Pro^{36}, Pro^{37}, Pro^{38} [$Trp(O_2)^{25}$, Asp^{28}]Exendin-4(1-39) -NH_2,
des Pro^{36}, Pro^{37}, Pro^{38} [$Trp(O_2)^{25}$, Asp^{28}]Exendin-4(1-39)-$(Lys)_6$-NH_2,
H-$(Lys)_6$-des Pro^{36}, Pro^{37}, Pro^{38} [$Trp(O_2)^{25}$, Asp^{28}]Exendin-4(1-39)-$(Lys)_6$-NH_2,
H-Asn-(Glu)5-des Pro^{36}, Pro^{37}, Pro^{38} [$Trp(O_2)^{25}$, Asp^{28}]Exendin-4(1-39)-$(Lys)_6$-NH_2,
H-$(Lys)_6$-des Pro^{36} [$Met(O)^{14}$, Asp^{28}]Exendin-4(1-39)-Lys_6-NH_2,
des $Met(O)^{14}$ Asp^{28} Pro^{36}, Pro^{37}, Pro^{38} Exendin-4(1-39) -NH_2,
H-$(Lys)_6$-des Pro^{36}, Pro^{37}, Pro^{38} [$Met(O)^{14}$, Asp^{28}]Exendin-4(1-39) -NH_2,
H-Asn-(Glu)5-des Pro^{36}, Pro^{37}, Pro^{38} [$Met(O)^{14}$, Asp^{28}] exendin-4(1-39) -NH_2,
des Pro^{36}, Pro^{37}, Pro^{38} [$Met(O)^{14}$, Asp^{28}]Exendin-4(1-39)-$(Lys)_6$-NH_2,
H-$(Lys)_6$-des Pro^{36}, Pro^{37}, Pro^{38} [$Met(O)^{14}$, Asp^{28}]Exendin-4(1-39)-Lys_6-NH_2,
H-Asn-$(Glu)_5$-des Pro^{36}, Pro^{37}, Pro^{38} [$Met(O)^{14}$, Asp^{28}] exendin-4(1-39)-(Lys)6-NH2,
H-$(Lys)_6$-des Pro^{36} [$Met(O)^{14}$, $Trp(O_2)^{25}$, Asp^{28}]Exendin-4(1-39)-Lys_6-NH_2,
des Asp^{28} Pro^{36}, Pro^{37}, Pro^{38} [$Met(O)^{14}$, $Trp(O_2)^{25}$]Exendin-4(1-39)-NH_2,
H-$(Lys)_6$-des Pro^{36}, Pro^{37}, Pro^{38} [$Met(O)^{14}$, $Trp(O_2)^{25}$, Asp^{28}]Exendin-4(1-39) -NH_2,
H-Asn-(Glu)5-des Pro^{36}, Pro^{37}, Pro^{38} [$Met(O)^{14}$, Asp^{28}] exendin-4(1-39) -NH_2,
des Pro^{36}, Pro^{37}, Pro^{38} [$Met(O)^{14}$, $Trp(O_2)^{25}$, Asp^{28}]Exendin-4(1-39)-$(Lys)_6$-NH_2,
H-$(Lys)_6$-des Pro^{36}, Pro^{37}, Pro^{38} [$Met(O)^{14}$, $Trp(O_2)^{25}$, Asp^{28}]Exendin-4(1-39)-$(Lys)_6$-NH_2,
H-Asn-$(Glu)_5$-des Pro^{36}, Pro^{37}, Pro^{38} [$Met(O)^{14}$, $Trp(O_2)^{25}$, Asp^{28}]Exendin-4(1-39)-$(Lys)_6$-NH_2,
eller et farmakologisk tolererbart salt derav.

12. Farmasøytisk formulering ifølge krav 9, som ytterligere omfatter Arg^{34}, Lys^{26} (N^{ε}(γ-glutamyl(N^{α}-heksadekanoyl))) GLP-1 (7-37) [liraglutid] eller et farmakologisk tolererbart salt derav.

13. Farmasøytisk formulering ifølge ett eller flere av kravene 1 til 12, i hvilken metionin er til stede i konsentrasjonsområdet opp til 10 mg/ml, fortrinnsvis opp til 3 mg/ml.

14. Fremgangsmåte for fremstilling av en formulering ifølge ett eller flere av kravene 1 til 13, i hvilken

(a) komponentene blir anbragt i en vandig løsning og

(b) pH-verdien blir justert.

15. Anvendelse av en formulering ifølge ett eller flere av kravene 1 til 13. til fremstilling av et legemiddel til behandling av diabetes mellitus.

16. Legemiddel til anvendelse i behandlingen av diabetes mellitus bestående av en formulering ifølge ett eller flere av kravene 1 til 13.

Figure 1

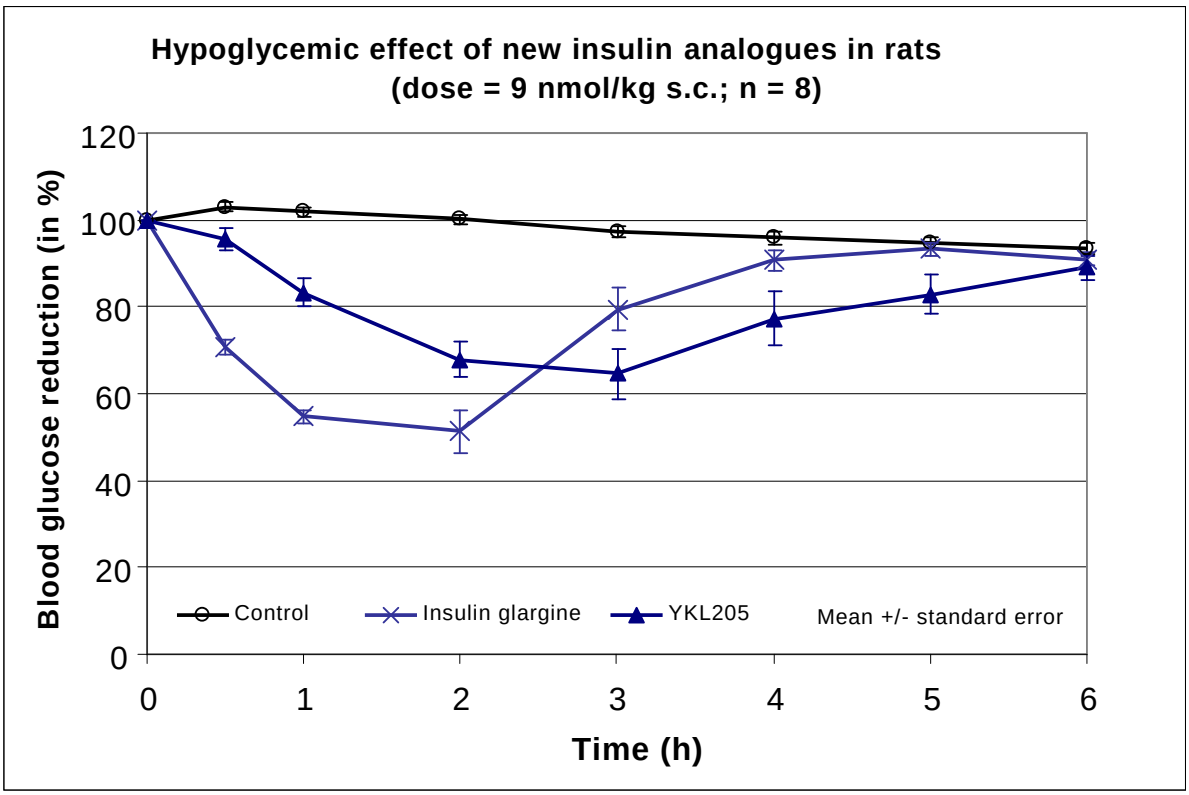

Hypoglycemic effect of new insulin analogues in rats
(dose = 9 nmol/kg s.c.; n = 8)
Blood glucose reduction (in %)
120
100
80
60
40
20
0
0
1
2
3
4
5
6
Time (h)
Control
Insulin glargine
YKL205
Mean +/- standard error

Figure 2

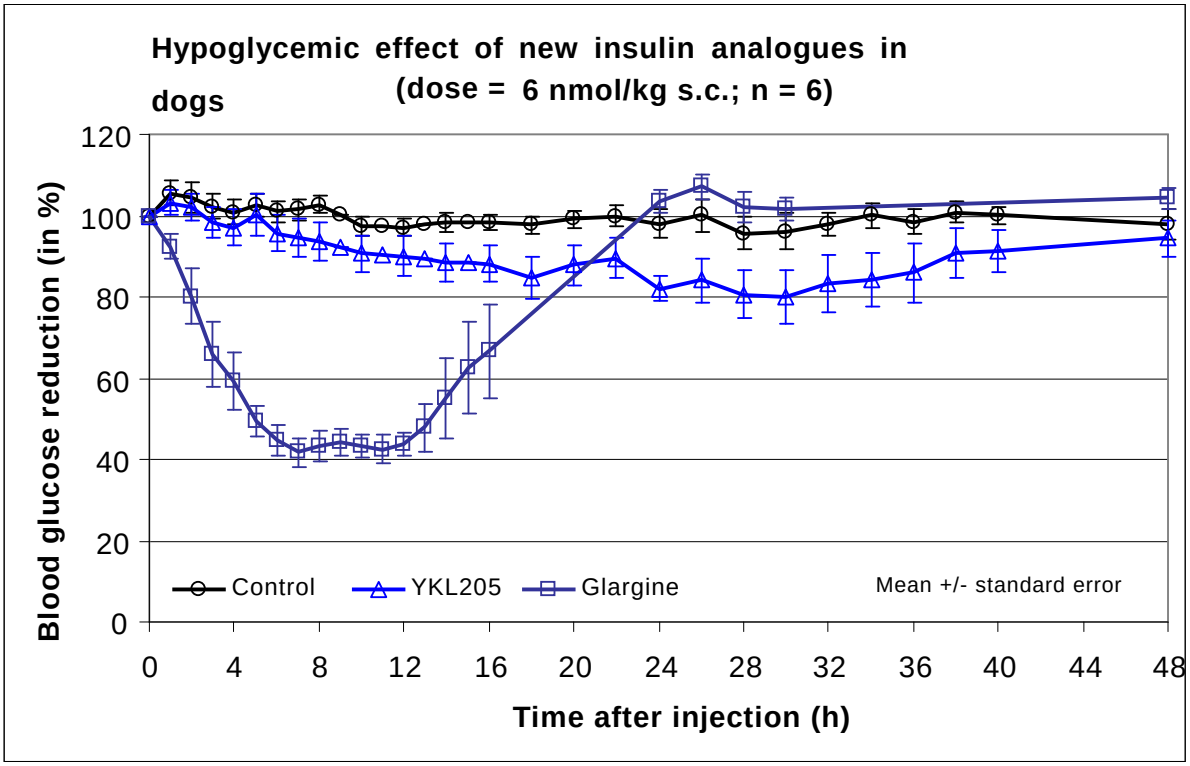

Hypoglycemic effect of new insulin analogues in dogs
(dose = 6 nmol/kg s.c.; n = 6)
Blood glucose reduction (in %)
120
100
80
60
40
20
0
0
4
8
12
16
20
24
28
32
36
40
44
48
Time after injection (h)
Control
YKL205
Glargine
Mean +/- standard error

Figure 3

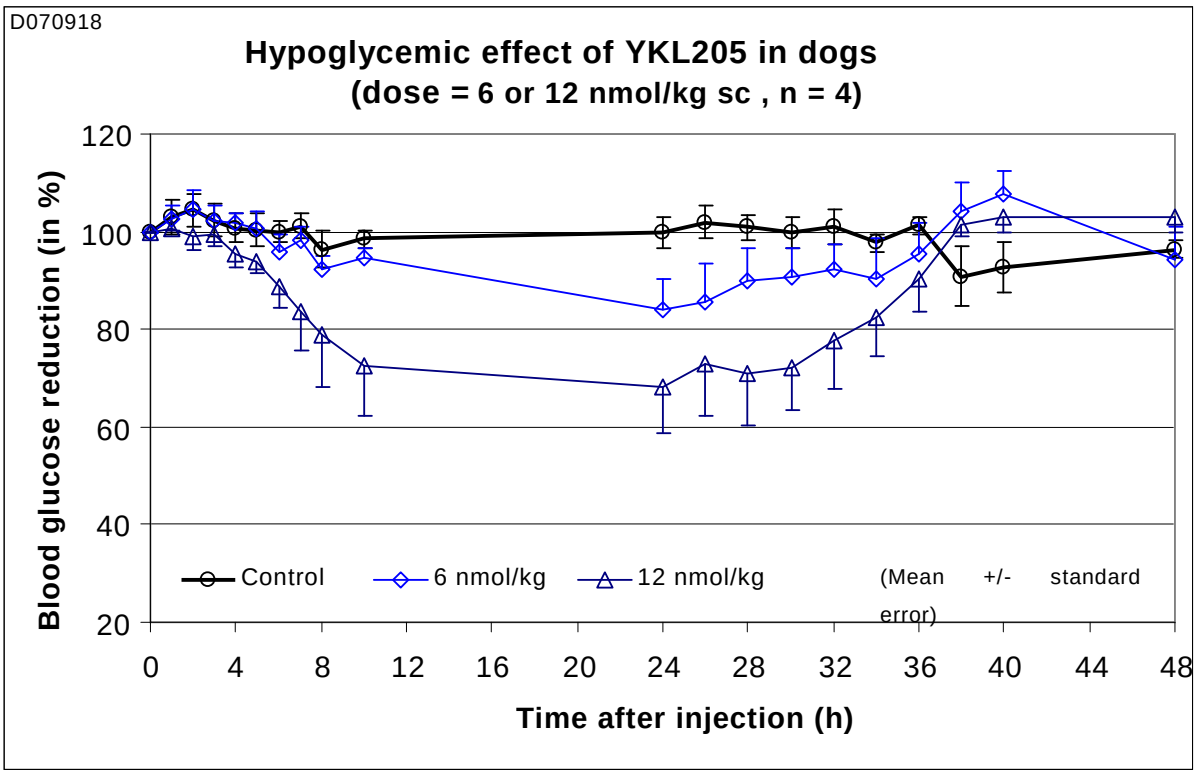

D070918
Hypoglycemic effect of YKL205 in dogs
(dose = 6 or 12 nmol/kg sc , n = 4)
Blood glucose reduction (in %)
120
100
80
60
40
20
0
4
8
12
16
20
24
28
32
36
40
44
48
Time after injection (h)
Control
6 nmol/kg
12 nmol/kg
(Mean +/- standard error)

Figure 4

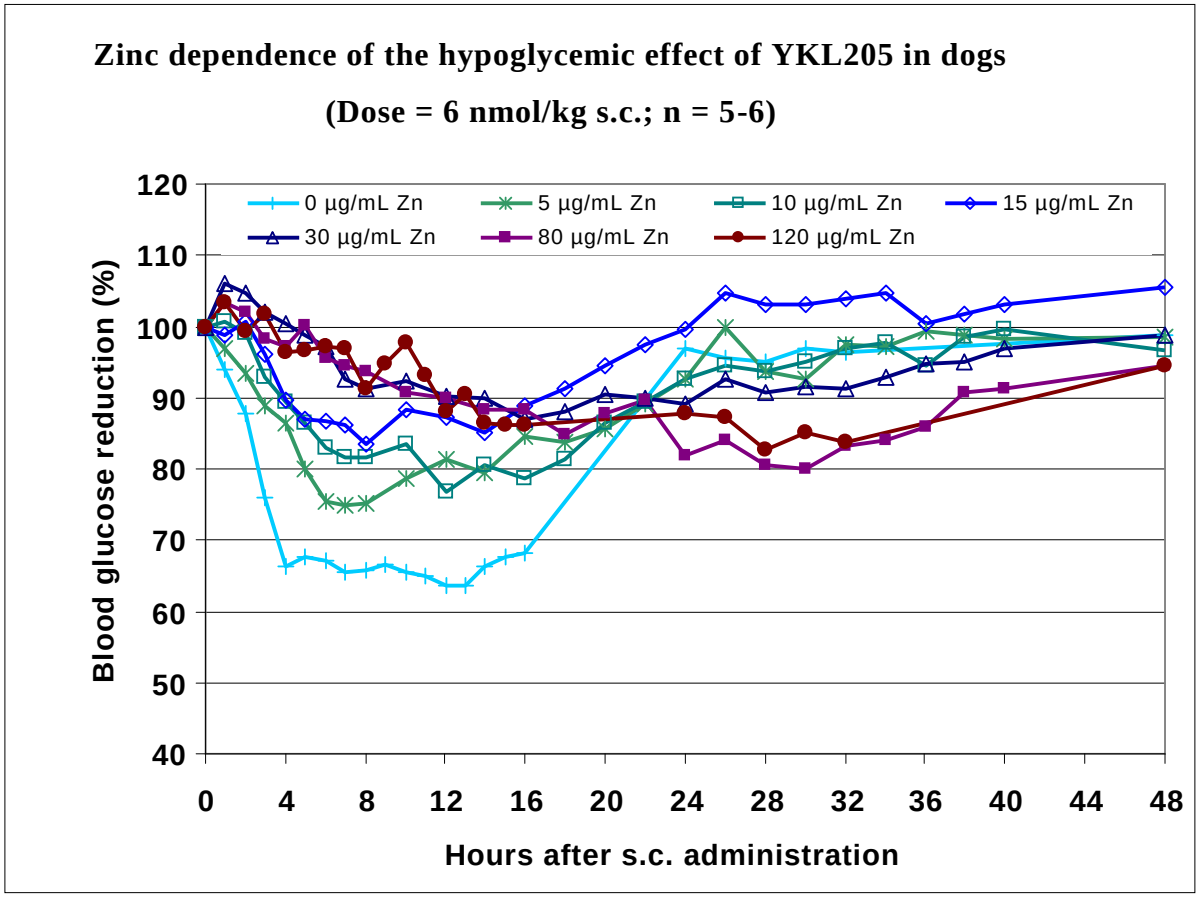

Zinc dependence of the hypoglycemic effect of YKL205 in dogs
(Dose = 6 nmol/kg s.c.; n = 5-6)
0 µg/mL Zn
5 µg/mL Zn
10 µg/mL Zn
15 µg/mL Zn
30 µg/mL Zn
80 µg/mL Zn
120 µg/mL Zn
Blood glucose reduction (%)
120
110
100
90
80
70
60
50
40
0
4
8
12
16
20
24
28
32
36
40
44
48
Hours after s.c. administration

Figure 5

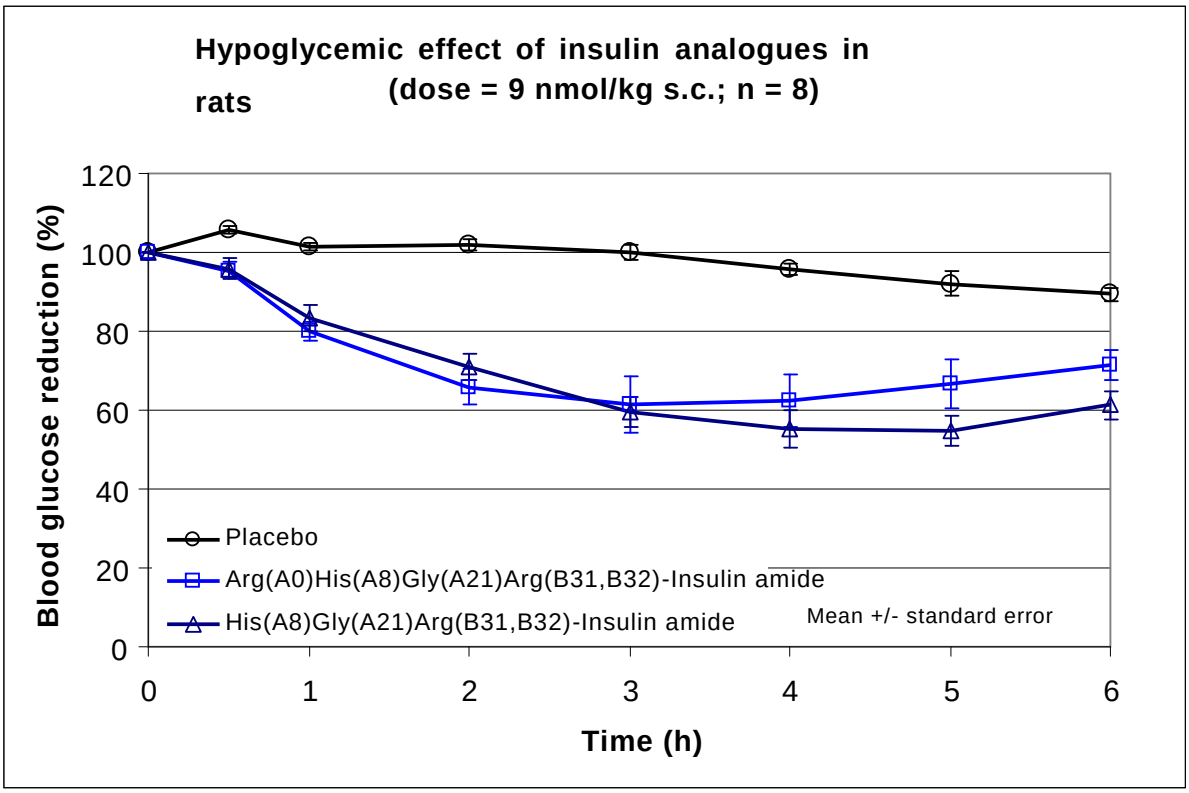
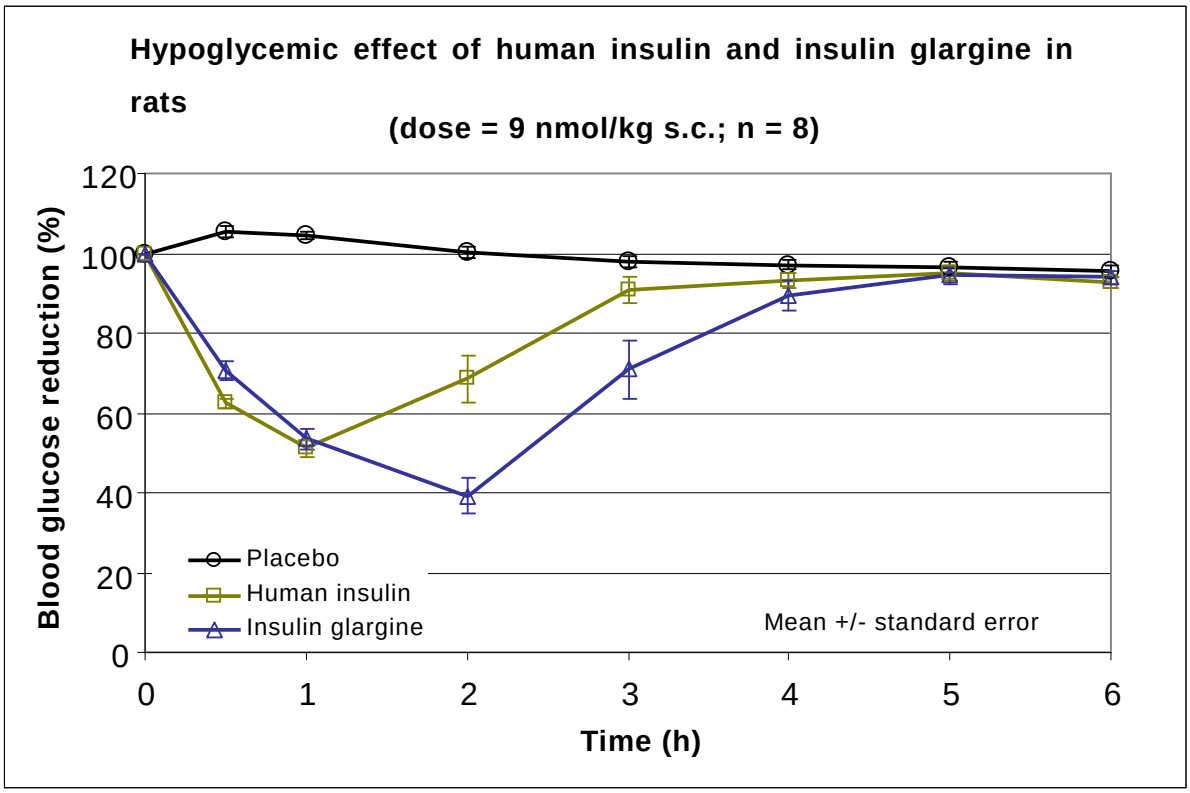

Hypoglycemic effect of insulin analogues in rats
(dose = 9 nmol/kg s.c.; n = 8)
Blood glucose reduction (%)
120
100
80
60
40
20
0
0
1
2
3
4
5
6
Time (h)
Placebo
Arg(A0)His(A8)Gly(A21)Arg(B31,B32)-Insulin amide
His(A8)Gly(A21)Arg(B31,B32)-Insulin amide
Mean +/- standard error

Figure 6


Hypoglycemic effect of human insulin and insulin glargine in rats
(dose = 9 nmol/kg s.c.; n = 8)
Blood glucose reduction (%)
120
100
80
60
40
20
0
0
1
2
3
4
5
6
Time (h)
Placebo
Human insulin
Insulin glargine
Mean +/- standard error