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(54) Benevnelse **PHARMACEUTICAL COMPOSITION COMPRISING desPro36Exendin-4(1-39)-Lys6-NH2 AND METHIONINE**

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Description

PHARMACEUTICAL COMPOSITION COMPRISING desPro36Exendin-4(1-39)-Lys6-NH2 AND METHIONINE

The present invention relates to a liquid composition comprising desPro^{36}Exendin-4(1-39)-Lys$_6$-NH$_2$ or/and a pharmacologically tolerable salt thereof and, optionally, at least one pharmaceutically acceptable excipient, characterized in that it comprises methionine and is free of EDTA and histidine and in that the composition comprises desPro^{36}Exendin-4(1-39)-Lys$_6$-NH$_2$ in an amount of from 0.01 mg/ml to 1.5 mg/ml.

The present application further relates to the composition according to the present invention for treating diabetes mellitus. The present application further relates to the use of a composition according to the present invention in the manufacture of a pharmaceutical for treating diabetes mellitus. The present application further relates to a method for manufacturing a composition according to the present invention, comprising formulating a GLP-1 agonist or/and a pharmacologically tolerable salt thereof with methionine and, optionally, at least one pharmaceutically acceptable excipient. The present application further relates to a method for treating a patient with a composition according to the present invention, comprising administering the composition to the patient.

Customary compositions of GLP-1 compounds comprise an isotonizing agent, a buffer for adjusting the pH, and a preservative.

WO2001/04156 (Zealand Pharmaceuticals) discloses a liquid composition of Ser^{39}-Exendin-4(1-39)-NH$_2$), sodium dihydrogen phosphate, and preservatives.

WO 2004/035623 (Zealand Pharmaceuticals) discloses a liquid composition comprising a stabilized Exendin, 50 mM histidine, 100 to 200 mM sucrose, mannitol or other acceptable sugar, 20 mM methionine, 20 mM asparagine-glutamine or Asp, at a pH of 5.3. Stabilization is effected by certain modifications of the amino acid building blocks of Exendin-4(1-39), for example, at positions Gln13, Met14, Trp25, or Asn28.

WO 2005/021022 (Novo Nordisk) discloses a liquid composition comprising acetylated GLP-1, phenol as a preservative, mannitol and glycerol as an isotonizing agent, and, optionally, a buffer.

WO 2006/051110 (Novo Nordisk) discloses liquid compositions comprising liraglutide (GLP-1 compound), poloxamer 188 or poloxamer 407 (Pluronic F-127) as a surface-active substance, phenol, propylene glycol, and sodium phosphate (pH 7.7). Addition of poloxamer-188 or poloxamer-407 led to stabilization.

US 2008/260840 discloses suspension formulations of an insulinotropic peptide.

US 2008/146490 discloses a pre-formulation, comprising a mixture of low viscosity, which forms a liquid crystalline phase after contact with an aqueous fluid. The mixture of low viscosity comprises a neutral diacyl lipid and/or tocopherol, a phospholipid, a biocompatible organic solvent and a GLP-1 analog.

US 2001/012829 discloses formulations comprising a stabilized GLP-1 compound to which a lipophilic substituent is attached.

WO 2005/028516 discloses plasma-protein affinity tags as system for administering active substances.

WO 2009/102467 discloses devices, formulations and methods for administering active substances.

WO 2010/044867 discloses highly concentrated active-substance particles and formulations and suspensions thereof.

CN101366692 discloses an Exendin-4 composition.

Exendins are a group of peptides which can lower blood glucose concentrations. Exendins have a certain similarity to the sequence of GLP-1 (7-36) (53%, Goke et al. J. Biol Chem 268, 19650-55). Exendin-3 and Exendin-4 stimulate an increase in

cellular cAMP production in the acinar cells of the guinea pig pancreas by interacting with Exendin receptors (Raufman, 1996, Reg. Peptides 61:1-18). Exendin-3, in contrast to Exendin-4, effects an increase in the release of amylase in the acinar cells of the pancreas. Exendins act as GLP-1 agonists.

Glucagon-like peptide 1 (GLP-1) is an endocrine hormone which enhances the insulin response following oral intake of glucose or fat. In general, GLP-1 lowers glucagon concentrations, slows gastric emptying, stimulates (pro)insulin biosynthesis, enhances sensitivity to insulin, and stimulates insulin-independent glycogen biosynthesis (Holst (1999), Curr. Med. Chem 6:1005, Nauck et al. (1997) Exp Clin Endocrinol Diabetes 105: 187, Lopez-Delgado et al. (1998) Endocrinology 139:2811). Human GLP-1 has 37 amino acid residues (Heinrich et al., Endocrinol. 115:2176 (1984), Uttenthal et al., J Clin Endocrinol Metabol (1985) 61:472). Active fragments of GLP-1 include GLP-1 (7-36) and GLP-1 (7-37).

Exendin-3, Exendin-4 and Exendin agonists have been proposed for treating diabetes mellitus and preventing hyperglycemia, by reducing gastric motility and gastric emptying (US 5,424,286 and WO98/05351).

Exendin analogs can be characterized by amino acid substitutions and/or C-terminal truncation of the native Exendin-4 sequence. Such Exendin analogs are described in WO 99/07404, WO 99/25727, and WO 99/25728.

Solid-phase synthesis of AVE0010 is described in WO 01/04156 A1. AVE0010 has the sequence: desPro^{36}Exendin-4(1-39)-Lys$_6$-NH$_2$. This substance is published as SEQ ID NO:93 in WO 01/04156:

H-G-E-G-T-F-T-S-D-L-S-K-Q-M-E-E-E-A-V-R-L-F-I-E-W-L-K-N-G-G-P-S-S-G-A-P-P-S-K-K-K-K-K-K-NH$_2$ (SEQ ID NO: 1)

Exendin-4 (39 AA) has the sequence:

H-G-E-G-T-F-T-S-D-L-S-K-Q-M-E-E-E-A-V-R-L-F-I-E-W-L-K-N-G-G-P-S-S-G-A-P-P-P-S-NH$_2$ (SEQ ID NO: 2)

Exendin-3 has the sequence (J. Bio. Chem., 267, 1992, 7402-7405):

H-His-Ser-Asp-Gly-Thr-Phe-Thr-Ser-Asp-Leu-Ser-Lys-Gln-Met-Glu-Glu-Glu-Ala-Val-Arg-Leu-Phe-Ile-Glu-Trp-Leu-Lys-Asn-Gly-Gly-Pro-Ser-Ser-Gly-Ala-Pro-Pro-Pro-Ser-NH_2 (SEQ ID NO: 3)

GLP-1 has the sequence:

H-A-E-G-T-F-T-S-D-V-S-S-Y-L-E-G-Q-A-A-K-E-F-I-A-W-L-V-K-G-R-NH_2 (SEQ ID NO: 4)

It is an object of the present invention to increase the stability of liquid formulations comprising AVE0010. More particularly, it is an object of the present invention to improve physical and chemical integrity. This object is achieved by formulating AVE0010 with methionine.

It was found that methionine can increase the storage stability of a composition comprising AVE0010. Methionine does not affect the physical integrity of these compositions.

It was found that, surprisingly, the addition of methionine is able to improve the storage stability of a composition according to the present invention by reducing the proportion of oxidation products of methionine, of proteins of high molecular weight, and of total impurities. These parameters are, individually or together, a measure of the chemical integrity of the compositions.

It was further found that, surprisingly, the biological activity of the compositions according to the present invention was increased by the addition of methionine.

The stability of pharmaceutically active polypeptides can be impaired by various mechanisms. These include pH, temperature, light, and the effects of certain constituents.

A range of customary constituents of formulations of GLP-1 agonists can be disadvantageous for the chemical or/and physical integrity and the storage stability of formulations which comprise a GLP-1 agonist. These are, for example, polysorbate 20, polysorbate 80, poloxamer 188, benzalkonium chloride, and lysine. The compositions according to the present invention are therefore preferably free of these constituents.

The present invention provides for a liquid composition comprising AVE0010 or/and a pharmacologically tolerable salt thereof and, optionally, at least one pharmaceutically acceptable excipient, wherein the composition is characterized in that it comprises methionine.

The composition according to the present invention preferably comprises methionine in an amount ranging from 0.5 mg/ml to 20 mg/ml, more preferably in an amount ranging from 1 mg/ml to 5 mg/ml. Methionine in the D-form can be used. Likewise, methionine in the L-form can be used. Likewise, mixtures of the D-form and the L-form in any desired proportions can be used.

More particularly, the composition according to the present invention is free of surfactants, such as polyols and partial and fatty acid esters and ethers of polyhydric alcohols such as those of glycerol and sorbitol. The compositions according to the present invention are more particularly free of partial and fatty acid esters and ethers of glycerol and sorbitol selected from a group comprising Span®, Tween®, Myrj®, Brij®, Cremophor®. Furthermore, the compositions according to the present invention are more particularly free of polyols selected from the group consisting of polypropylene glycols, polyethylene glycols, poloxamers, Pluronics, Tetronics. More particularly, the composition according to the present invention is free of at least one substance selected from polysorbate and poloxamer.

More particularly, the composition according to the present invention is substantially free, preferably free, of polysorbate, such as, for example, polysorbate 20.

More particularly, the composition according to the present invention is substantially free, preferably free, of polysorbate 80.

More particularly, the composition according to the present invention is substantially free, preferably free, of poloxamer, such as, for example, poloxamer 188.

More particularly, the composition according to the present invention is substantially free, preferably free, of benzalkonium chloride.

The composition according to the present invention is free of histidine.

The composition according to the present invention is free of EDTA, more particularly sodium EDTA.

The composition according to the present invention can comprise one or more substances which are customarily used to buffer the pH (buffer substances). Examples of such buffer substances are acetate, citrate, and phosphate, for example, in amounts of up to 5 mg/ml, up to 4 mg/ml, up to 3 mg/ml, or up to 2 mg/ml.

The composition according to the present invention can, likewise, be substantially free of buffer substances. Likewise, the composition according to the present invention can be free of buffer substances.

The invention according to the present invention can be substantially free of citrate, acetate, and/or phosphate, or else free of citrate, acetate, and/or phosphate.

More particularly, the composition according to the present invention is substantially free, preferably free, of histidine and sodium EDTA.

More particularly, no insulin is present in the composition according to the present invention.

The pharmaceutical composition of the present invention can have an acidic or physiological pH. An acidic pH range is preferably in the range of pH 1-6.8, pH 3.5-6.8, or pH 3.5-5. A physiological pH is preferably in the range of pH 2.5-8.5, more preferably pH 4.0 to 8.5, even more preferably pH 6.0 to 8.5. Especially preferred is a pH of approximately 4.5. For pH adjustment, physiologically safe dilute acids (typically HCl) and alkalis (typically NaOH) are suitable.

The composition according to the present invention can comprise a suitable preservative. Suitable preservatives are, for example, phenol, m-cresol, benzyl alcohol, and/or p-hydroxybenzoates. m-Cresol is preferred.

Furthermore, the composition according to the present invention can comprise suitable isotonizing agents. Suitable isotonizing agents are, for example, glycerol, dextrose, lactose, sorbitol, mannitol, glucose, NaCl, calcium or magnesium compounds such as $CaCl_2$ etc. The concentrations of glycerol, dextrose, lactose, sorbitol, mannitol, and glucose are customarily in the range of 100-250 mM, NaCl in a concentration of up to 150 mM. Glycerol is preferred.

More particularly, the composition is intended for parenteral administration. The composition according to the present invention is preferably an injectable composition, more preferably for subcutaneous injection. More particularly, the composition of the present invention is suitable for injection once a day.

More particularly, the formulation according to the present invention has, after storage for 1 month, 2 months, 4 months, or 6 months at a temperature of + 5°C or 25°C, an activity of at least 80%, at least 90%, at least 95%, or at least 98% of the activity at the start of storage.

In the present application, “activity” means the activity of the GLP-1 agonist which is used in the formulation according to the present invention. Methods for determining the activity of a GLP-1 agonist are known to a person skilled in the art.

Preferably, the composition according to the present invention has a biological activity of GLP-1 agonist of at least 89% or at least 90% after storage for 6 months

at 25°C. The composition according to the present invention preferably has a biological activity of GLP-1 agonist of at least 45% or at least 50% after storage for 6 months at 40°C. The GLP-1 agonist is AVE0010 (lixisenatide, H-desPro^{36}-Exendin-4-Lys$_6$-NH$_2$).

More particularly, the formulation according to the present invention exhibits chemical integrity after storage for 1 month, 2 months, 3 months, 4 months, or 6 months. Chemical integrity means, more particularly, that after storage at a temperature of +5°C, 25°C, or 40°C the formulation comprises at least 80%, at least 90%, at least 95%, or at least 98% of the active substance, compared with the start of storage, in a substantially chemically unchanged form.

Chemical integrity can mean the chemical integrity of the GLP-1 agonist. GLP-1 agonists may comprise a methionine residue (e.g. position 14 in AVE0010). Chemical integrity of the GLP-1 agonist means, more particularly, that oxidation of this methionine residue is prevented. Here, chemical integrity means, more particularly, that the proportion of oxidized methionine with respect to the entire methionine content of the GLP-1 agonist after storage for 1, 2, 3, 4, or 6 months is below 0.7%, below 0.6%, below 0.5%, below 0.4%, or below 0.3%. Storage can be effected, for example, at 5°C, 25°C, or 40°C. Storage for 6 months at 5°C is preferred, in which case the proportion of oxidized methionine is below 0.3%. Likewise, storage for 6 months at 25°C is preferred, in which case the proportion of oxidized methionine is below 0.7%, below 0.6%, below 0.5%, below 0.4%, or below 0.3%. is. Likewise, storage for 6 months at 40°C is preferred, in which case the proportion of oxidized methionine is below 1%, below 0.7%, below 0.6%, below 0.5%, below 0.4%, or below 0.3%.

Chemical integrity can mean a very low proportion of total impurities in the formulation according to the present invention. The proportion of total impurities with respect to the entire mass of the GLP-1 agonist present in the formulation after storage for 6 months at 40°C is more particularly below 50%, below 10% after storage at 25°C, or/and below 1.8% after storage at 5°C.

Chemical integrity can mean a very low proportion of proteins of high molecular weight in the formulation according to the present invention. The proportion of proteins of high molecular weight with respect to the entire mass of the GLP-1 agonist present in the formulation after storage for 6 months at 40°C is more particularly below 5%, below 4%, below 3%, or below 2%. After storage for 6 months at 25°C, the proportion of proteins of high molecular weight with respect to the entire mass of the GLP-1 agonist present in the formulation is more particularly below 0.8%, below 0.7%, or below 0.6%.

More particularly, the formulation according to the present invention exhibits physical integrity after storage for 1 month, 2 months, 4 months, or 6 months. Physical integrity means, more particularly, that after storage at a temperature of + 5°C, 25°C, or 40°C the formulation comprises at least 80%, at least 90%, at least 95%, or at least 98% of the active substance, compared with the start of storage, in a substantially physically unchanged form.

Physical integrity can mean the integrity of the GLP-1 agonist. Physical integrity means, more particularly, that the GLP-1 agonist does not form aggregates, such as, for example, fibrils.

The sequences of AVE0010 (SEQ ID NO:1), Exendin-4 (SEQ ID NO: 2), and Exendin-3 (SEQ ID NO:3) show a high degree of similarity. The sequences of AVE0010 and Exendin-4 are identical at positions 1-37. Sequence 1-39 from Exendin-4 is at 37 of the 39 positions (94%) identical to the Exendin-3 sequence at positions 48-86. With reference to the sequences, a person skilled in the art can readily convert the positions specified herein, which relate to a particular sequence (e.g. to the sequence of AVE0010 or Exendin-4), to other sequences.

Pharmaceutically tolerable salts can be manufactured in a further method step after completion of the synthesis cycles of the method according to the present invention. The manufacture of pharmaceutically tolerable salts of peptides is known to a person skilled in the art. A preferred pharmaceutically tolerable salt is acetate.

Likewise, pharmacologically tolerable salts of AVE0010 are preferred.

AVE0010 is used in an amount ranging from 0.01 mg/ml to 0.5 mg/ml or 0.05 mg/ml to 1.5 mg/ml.

In a particular embodiment, the formulation according to the present invention comprises the following constituents:

(a) desPro^{36}Exendin-4(1-39)-Lys$_6$-NH$_2$(e.g. approximately 0.1 mg/ml),

(b) sodium acetate trihydrate (approximately 3.5 mg/ml),

(c) m-cresol (approximately 2.7 mg/ml),

(d) L-methionine (approximately 3 mg/ml),

(e) 85% glycerol (approximately 18 mg/ml),

(f) approximately 0.1 N hydrochloric acid, if adjustment to a pH of approximately 4.5 is required,

(g) approximately 0.1 N NaOH solution, if adjustment to a pH of approximately 4.5 is required, and

(h) water.

More particularly, the formulation according to the present invention consists of the constituents (a) to (h) mentioned.

In the present application, “approximately” means that the constituents can be present, for example, within the ranges of ±10, ±20, or ±30 around the specified values in the compositions according to the present invention.

Suitable packaging for the composition according to the present invention is, for example, a syringe or a glass vessel with a suitable closure, from which individual therapeutically effective doses can be withdrawn as needed. Equally suitable are injection pens for administering doses; such pens comprise a container (e.g. a cartridge) which contains a pharmaceutical composition according to the present invention.

The present invention provides for a method for treating a patient with a composition according to the present invention, comprising administering the composition to the patient.

The composition according to the present invention is intended more particularly for use in treating diabetes mellitus, more particularly for treating type I or type II diabetes mellitus. Further possible indications are symptoms which are associated with diabetes mellitus. Preferably, the composition according to the present invention is used to control the fasting, postprandial, or/and postabsorptive plasma glucose concentration, to improve glucose tolerance, to prevent hypoglycemia, to prevent functional loss of the β-cells of the pancreas, to effect weight loss, or/and to prevent weight gain.

The present invention further provides for the use of a composition according to the present invention in the manufacture of a pharmaceutical for treating diabetes mellitus, more particularly type I or type II, or/and the symptoms associated with it, as described herein.

The present invention further provides a method for manufacturing a composition according to the present invention, comprising formulating the AVE0010 GLP-1 agonist or/and a pharmacologically tolerable salt thereof with methionine and, optionally, at least one pharmaceutically acceptable excipient.

The composition according to the present invention can be used together with the administration of metformin, sulfonylurea, or glitazones, a long-acting insulin/insulin derivative, and/or a combination thereof, more particularly as an add-on therapy in the administration of metformin.

The composition according to the present invention can be used in patients whose blood sugar levels cannot be controlled or adjusted sufficiently by the administration of metformin, sulfonylurea, or glitazones, a long-acting insulin/insulin derivative, and/or a combination thereof.

The composition according to the present invention can be used in patients with type II diabetes as additive to a diet in order to improve blood sugar control.

More particularly, the composition comprises desPro^{36}Exendin-4(1-39)-Lys$_6$-NH$_2$ (AVE0010), liraglutide and/or a pharmacologically tolerable salt together with methionine and/or a pharmacologically tolerable salt.

More particularly, Lantus®, N$^{\varepsilon B29}$–tetradecanoyl des (B30) human insulin, or Insuman® is useful as a long-acting insulin derivative.

Especially preferred is the composition according to the present invention for use in an add-on therapy with metformin and/or a long-acting insulin/insulin derivative and/or a pharmacologically tolerable salt thereof for treating type II diabetes and/or obesity, more particularly in patients who are younger than 50 years old and/or have a body mass index of at least 30.

The composition according to the present invention is more particularly for use in an add-on therapy for the treatment of type II diabetes with metformin and AVE0010. Metformin and AVE0010 can be administered in a time interval of 24 hours. Metformin and AVE0010 can each be administered in a one-a-day dosage. Metformin and AVE0010 can be administered by means of different routes of administration. Metformin can be administered orally, AVE0010 subcutaneously.

Patients treated with the composition according to the present invention can have an HbA1c value in the range of 7% to 10%. They are preferably in the age range of 18 to 50 years old.

The composition according to the present invention for use in an add-on therapy is more particularly applicable to patients in whom type II diabetes cannot be sufficiently controlled with metformin alone.

More particularly, metformin is administered as follows: at least 1.0 g/day, preferably at least 1.5 g/day for 3 months.

The invention is further elucidated by the following examples and figures.

Legends

FIGS. 1 and 2 show the percentage content of oxidized methionine Met(ox) with respect to the entire methionine content of AVE0010 after storage at different temperatures. 1: start of storage t0. 2: storage for 1 month. 3: storage for 3 months. 3: storage for 6 months. FIG. 1: batch 894. FIG. 2: batch 897.

FIGS. 3 and 4 show the percentage content of protein impurities of high molecular weight (with respect to AVE0010) after storage at different temperatures. 1: start of storage t0. 2: storage for 1 month. 3: storage for 3 months. 3: storage for 6 months. FIG. 3: batch 894. FIG. 4: batch 897.

FIGS. 5 and 6 show the percentage content of all impurities (with respect to AVE0010) after storage at different temperatures. 1: start of storage t0. 2: storage for 1 month. 3: storage for 3 months. 3: storage for 6 months. FIG. 5: batch 894. FIG. 6: batch 897.

Example 1

Liquid composition comprising AVE0010 and methionine

The purpose of the study is the evaluation of the chemical or/and physical stability of formulations of AVE0010 (solution for injection, 0.1 mg/ml) with and without methionine, when the product is stored in cartridges under long-term conditions and accelerated conditions for up to 6 months.

The following compositions are tested:

Composition A

(2 parallel batches: AVE0010_09_894_A and AVE0010_09_897_A)

Substance	Specification according to pharmacopeia	Amount per unit
AVE0010	Sanofi-Aventis	0.10 mg
Sodium acetate trihydrate	Ph. Eur./USP	3.50 mg
m-Cresol	Ph. Eur./USP	2.70 mg
85% Glycerol	Ph. Eur./USP	18.00 mg
0.1N Hydrochloric acid	Ph. Eur./USP	ad pH 4.5
0.1N NaOH solution	Ph. Eur./USP	ad pH 4.5
Water for injection (WfI)	Ph. Eur./USP	ad 1.0 ml

Composition B

(2 parallel batches: AVE0010_09_894_B and AVE0010_09_897_B)

Substance	Specification according to pharmacopeia	Amount per unit
AVE0010	Sanofi-Aventis	0.10 mg
Sodium acetate trihydrate	Ph. Eur./USP	3.50 mg
m-Cresol	Ph. Eur./USP	2.70 mg
L-Methionine	Ph. Eur./USP	3.00 mg
85% Glycerol	Ph. Eur./USP	18.00 mg
0.1N Hydrochloric acid	Ph. Eur./USP	ad pH 4.5
0.1N NaOH solution	Ph. Eur./USP	ad pH 4.5
Water for injection (WfI)	Ph. Eur./USP	ad 1.0 ml

The formulations are stored in units which are intended for clinical studies and for sales and distribution.

Term	Description
Injection cartridge	Cartridge, 3 ml colorless, type I glass (Ph. Eur.), SAP number 100922
Crimped lid and inserted within a gray sealing disk	7.5 mm Crimped lid: aluminum Sealing disk (exterior): isoprene rubber, material number 7773/35 Sealing disk (interior): bromobutyl rubber, material number 4780/40 Type I closure (Ph. Eur./USP) SAP number 164571
Plunger	9.2 × 11 mm Bromobutyl rubber, black SAP number 120521

5 Storage times, storage conditions, test time points are summarized in the following table.

Condition	Test interval (months)			
	0	1	3	6
Long-term storage				
+ 5 ± 3°C	X	X	X	X
Accelerated conditions (temperature, humidity)				
+ 25 ± 2°C/60 ± 5% RH		X	X	X
+ 40 ± 2°C/75 ± 5% RH		X	X	X

The formulations are stored horizontally. RH means relative humidity. Test time point 0 is the start of storage. The measurements at time point 0 are used as a

reference for all conditions tested. During the tests, the samples are stored at + 5 ± 3°C.

The physical and chemical stability of the stored formulations is determined with the help of the following tests:

- Description
- Clarity of the solution and color thereof
- pH
- Chemical stability (purity and impurities, determined by HPLC, more particularly the proportion of oxidation products and of total impurities)
- Proteins of high molecular weight, determined by HPSEC
- Visible particles
- Biological activity of the formulations

Results

The formulations were studied separately for the parallel batches (894 and 897) with regard to the following parameters:

- **Biological activity of AVE0010.** At 5°C and 25°C, activity even after 6 months was at least 96% of initial activity. The activities of the compositions according to the present invention were greater than the activities of the comparative compositions. At 40°C, activity after 6 months in the absence of methionine was approximately 43%. In the presence of methionine, activity was approximately 51% and thus clearly greater than in the absence of methionine.

- **Oxidation products.** Measurements were carried out on an HPLC instrument (model: alliance) from Water Systems, using the 100% peak area method. For separation, a gradient of 0.1% TFA and acetonitrile as the mobile phase and a C18 reversed-phase column (Jupiter) as the stationary phase were used. At 5°C, the proportion of oxidized methionine Met(ox) in AVE0010 in the absence of methionine was 0.3%. At 25°C, the proportion was in the range of 0.6-0.8%, at 40°C 1.3%. When the formulation comprised methionine, the proportion of

oxidized methionine was distinctly lower. It was never more than 0.2% under all conditions tested. At 25°C, the proportion was thus approximately only ¼ to ⅓ of the content in the absence of methionine, even at 40°C approximately only ⅙ (see FIGS. 1 and 2).

- **Proteins of high molecular weight.** At 5°C, the proportion was between 0.1 and 0.3% and remained substantially unchanged during the entire storage time. At 25°C, the proportion rose in the absence of methionine to 0.9 and 1.3%, respectively. In the presence of methionine, the proportion was 0.4 to 0.5% and thus less than half as high. At 40°C, the proportion in the absence of methionine was 5.4% and 6.2%, respectively, while in the presence of methionine it was only 1.6 and 1.7%, respectively, and thus clearly lower (see FIGS. 3 and 4).

- **Total impurities.** At 5°C, total impurities rose over the storage time of 6 months slightly from 1.2 to 1.8 or 1.9% (absence of methionine). When methionine was present, the rise was a little lower. At 25°C, a rise to 10.6% and 11.8%, respectively, was observed. In the presence of methionine, the values were below 10%. At 40°C, the proportion rose up to 54% (without methionine). When methionine was present, the proportion was approximately only 47% (see FIGS. 5 and 6).

The percentage values are the content values (percentage values of impurities) of the oxidation products, of the total impurities, and of the higher-molecular-weight proteins (HMWP).

All values were determined by HPLC with the so-called 100% method. Here, in particular, it involves reversed-phase HPLC (C 18 column), in which a gradient method was used for the mobile phase:

a) 0.1% TFA, 15% ACN and b) 0.1% TFA, 75% ACN.

Detection at 215 nm (UV).

The high-molecular-weight proteins (HMWP) were detected by HPSEC, described in European Pharmacopeia 6.0 for injectable insulin preparations.

The data are summarized in the following tables:

Mean values: AVE0010_09_894_A + B								
	AVE0010_09_894_A				**AVE0010_09_894_B**			
5°C	t_0	1 Mon.	3 Mon.	6 Mon.	t_0	1 Mon.	3 Mon.	6 Mon.
Total impurities	1.2	1.5	2.1	1.8	1.1	1.3	1.5	1.7
AVE0010 Test	101.5	99.6	98.0	97.8	101.1	100.5	99.4	98.6
Proteins of high molecular weight	0.3	0.3	0.4	0.3	0.2	0.2	0.2	0.3
Oxidation products	0.3	0.4	0.4	0.3	0.1	0.2	0.1	0.1
25°C	t_0	1 Mon.	3 Mon.	6 Mon.	t_0	1 Mon.	3 Mon.	6 Mon.
Total impurities	1.2	3.0	6.4	11.8	1.1	2.5	5.7	9.8
AVE0010 Test	101.5	97.9	94.0	88.6	101.1	98.7	94.8	90.9
Proteins of high molecular weight	0.3	0.4	0.6	1.3	0.2	0.3	0.3	0.5
Oxidation products	0.3	0.4	0.5	0.8	0.1	0.2	0.2	0.2
40°C	t_0	1 Mon.	3 Mon.	6 Mon.	t_0	1 Mon.	3 Mon.	6 Mon.
Total impurities	1.2	13.4	34.3	54.1	1.1	12.1	30.4	46.8
AVE0010 Test	101.5	87.1	66.6	42.5	101.1	88.8	70.8	50.9
Proteins of high molecular weight	0.3	1.0	2.6	6.2	0.2	0.5	0.9	1.7

Oxidation products	0.3	0.6	0.9	1.3	0.1	0.2	0.2	0.2

Mean values: AVE0010_09_897_A + B								
	AVE0010_09_897_A				**AVE0010_09_897_B**			
5°C	t_0	1 Mon.	3 Mon.	6 Mon.	t_0	1 Mon.	3 Mon.	6 Mon.
Total impurities	1.2	1.6	1.8	1.9	1.0	1.3	1.5	1.7
AVE0010 Test	99.2	98.2	97.5	96.7	99.5	99.2	98.0	97.1
Proteins of high molecular weight	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.1
Oxidation products	0.3	0.3	0.3	0.3	0.1	0.1	0.1	0.1
25°C	t_0	1 Mon.	3 Mon.	6 Mon.	t_0	1 Mon.	3 Mon.	6 Mon.
Total impurities	1.2	3.3	6.7	10.6	1.0	2.7	5.8	9.1
AVE0010 Test	99.2	96.6	92.8	87.4	99.5	97.8	93.6	90.0
Proteins of high molecular weight	0.1	0.2	0.5	0.9	0.1	0.1	0.2	0.4
Oxidation products	0.3	0.4	0.5	0.6	0.1	0.1	0.2	0.2
40°C	t_0	1 Mon.	3 Mon.	6 Mon.	t_0	1 Mon.	3 Mon.	6 Mon.
Total impurities	1.2	13.1	33.5	53.9	1.0	11.8	29.8	47.0
AVE0010 Test	99.2	86.8	66.5	42.6	99.5	88.0	70.7	51.0
Proteins of high molecular weight	0.1	0.8	2.2	5.4	0.1	0.4	0.9	1.6
Oxidation products	0.3	0.5	0.8	1.3	0.1	0.1	0.2	0.2

Conclusion

The proportion of oxidation products, of proteins of high molecular weight, and of total impurities are, individually or together, a measure of the chemical integrity of the compositions. From the results described above with the example compositions, it follows that the liquid compositions according to the present invention comprising

- a GLP-1 agonist or/and a pharmacologically tolerable salt thereof (more particularly AVE0010 or/and a pharmacologically tolerable salt thereof),
- optionally at least one pharmaceutically acceptable excipient,
- and methionine,

have improved stability or/and chemical integrity. The proportion of oxidized methionine, of total impurities, and of proteins of high molecular weight is lower in the compositions according to the present invention than in the comparative compositions. The composition according to the present invention (batches 894_B and 897_B) and the comparative compositions (batches 894_A and 897_A) differ in the presence/absence of methionine. Therefore, improved stability or/and chemical integrity can be ascribed to the methionine constituent in compositions according to the present invention.

Example 2

In a further experiment, it was studied how sodium EDTA and histidine have an effect in a composition according to the present invention.

Composition B (as in example 1)

Substance	Specification according to pharmacopeia	Amount per unit
AVE0010	Sanofi-Aventis	0.10 mg

Substance	Specification according to pharmacopeia	Amount per unit
Sodium acetate trihydrate	Ph. Eur./USP	3.50 mg
m-Cresol	Ph. Eur./USP	2.70 mg
L-Methionine	Ph. Eur./USP	3.00 mg
85% Glycerol	Ph. Eur./USP	18.00 mg
0.1N Hydrochloric acid	Ph. Eur./USP	ad pH 4.5
0.1N NaOH solution	Ph. Eur./USP	ad pH 4.5
Water for injection (WfI)	Ph. Eur./USP	ad 1.0 ml

Composition C

Substance	Specification according to pharmacopeia	Amount per unit
AVE0010	Sanofi-Aventis	0.10 mg
Sodium acetate trihydrate	Ph. Eur./USP	3.50 mg
Sodium EDTA	Ph. Eur./USP	1.00 mg
m-Cresol	Ph. Eur./USP	2.70 mg
L-Methionine	Ph. Eur./USP	3.00 mg
L-Histidine	Ph. Eur./USP	3.10 mg
85% Glycerol	Ph. Eur./USP	18.00 mg
0.1N Hydrochloric acid	Ph. Eur./USP	ad pH 4.5
0.1N NaOH solution	Ph. Eur./USP	ad pH 4.5
Water for injection (WfI)	Ph. Eur./USP	ad 1.0 ml

In a standard experimental design, rabbits were treated with composition B or C or a saline solution subcutaneously (s.c.) or intramuscularly (i.m.). In each case, half the rabbits were sacrificed after 24 hours or 120 hours in order to determine the

acute or subacute effects of the administration histologically. Also, it was determined whether repair/regeneration of any changes occurred.

Following subcutaneous injection of composition C, the animals showed after 24 hours, in contrast to the saline control, a light to moderate inflammatory reaction in the subcutaneous connective tissue. After subcutaneous injection 120 hours earlier, a clear trend was observable for the observed changes to repair by a fibroblastic reaction. Thus, compatibility could still be rated as moderate (instead of as incompatible).

With composition B, the animals showed after subcutaneous injection no or minimal differences to the saline control (good compatibility).

After intramuscular injection of composition C, the animals exhibited muscular necrosis (multifocal or disseminated), clearly differing from the saline controls, in which only the site of injection was visible as a clearly circumscribed necrotic area. With composition C, mineralization of the necrotic muscular tissue was observed after 120 hours, visible even in a necropsy of the animals. Although small or focal mineralizations at various sites in rabbits are not unusual, the mineralizations after injection of composition C were clearly associated with the necrotic areas. Thus, the reversibility of the lesions caused by the injection is more than questionable. Based on these findings, composition C after intramuscular injection in rabbits was rated as incompatible.

Composition B after intramuscular injection showed good compatibility (no or minimal differences to the saline control).

From these data, it follows that composition B, compared to composition C, had an improved compatibility in intramuscular or subcutaneous administration. Subcutaneous injection is the preferred route of administration for the compositions comprising a GLP-1 agonist, more particularly AVE0010, described in this application.

Thus, the compositions according to the present invention, which comprise a GLP-1 agonist, more particularly AVE0010, can be free of EDTA or/and histidine. Likewise, the compositions according to the present invention can be substantially free of EDTA and histidine.

Patentkrav

1. Flytende sammensetning som omfatter desPro^{36}Exendin-4(1-39)-Lys$_6$-NH$_2$ og/eller et farmakologisk tolererbart salt derav og eventuelt minst én farmasøytisk akseptabel eksipiens, **karakterisert ved at** den inneholder metionin og er fri for EDTA og histidin og **ved at** sammensetningen inneholder desPro^{36}Exendin-4(1-39)-Lys$_6$-NH$_2$ i en mengde på fra 0,01 mg/ml til 1,5 mg/ml.

2. Flytende sammensetning ifølge krav 1, **karakterisert ved at** den inneholder et farmasøytisk akseptabelt konserveringsmiddel, nærmere bestemt m-kresol.

3. Flytende sammensetning ifølge krav 1 eller 2, **karakterisert ved at** den inneholder glyserol.

4. Flytende sammensetning ifølge ett av de foregående kravene, **karakterisert ved at** den har en pH fra 3,5 til 5.

5. Flytende sammensetning ifølge ett av de foregående kravene, **karakterisert ved at** den inneholder metionin i en mengde på fra 0,5 mg/ml til 20 mg/ml, fortrinnsvis i en mengde på fra 1 mg/ml til 5 mg/ml.

6. Flytende sammensetning ifølge ett av de foregående kravene, **karakterisert ved at** den omfatter de følgende bestanddelene:
(a) desPro^{36}Exendin-4-(1-39)-Lys$_6$-NH$_2$,
(b) natriumacetat,
(c) m-kresol,
(d) L-metionin,
(e) 85 % glyserol,
(f) ca. 0,1 N saltsyre, om nødvendig for å stille inn en pH på ca. 4,5,
(g) ca. 0,1 N NaOH-løsning, om nødvendig for å stille inn en pH på ca. 4,5 og
(h) vann.

7. Sammensetning ifølge ett av de foregående kravene for anvendelse i behandlingen av diabetes mellitus.

8. Sammensetning for anvendelsen ifølge krav 7 for behandling av diabetes type II.

9. Sammensetning for anvendelsen ifølge krav 7 eller 8, **karakterisert ved at** den administreres sammen med metformin, et sulfonylurea, et glitazon og/eller et langtidsvirkende insulin og/eller en kombinasjon derav og/eller et farmakologisk tolererbart salt derav.

10. Sammensetning for anvendelsen ifølge krav 9, **karakterisert ved at** administreringen er en tilleggsbehandling hos pasienter som ikke kunne oppnå tilstrekkelig blodsukkerkontroll med metformin, et sulfonylurea, et glitazon og/eller et langtidsvirkende insulin.

11. Sammensetning for anvendelsen ifølge krav 9 eller 10, **karakterisert ved at** de behandlede pasientene har en HbA1c-verdi på fra 7 til 10 %.

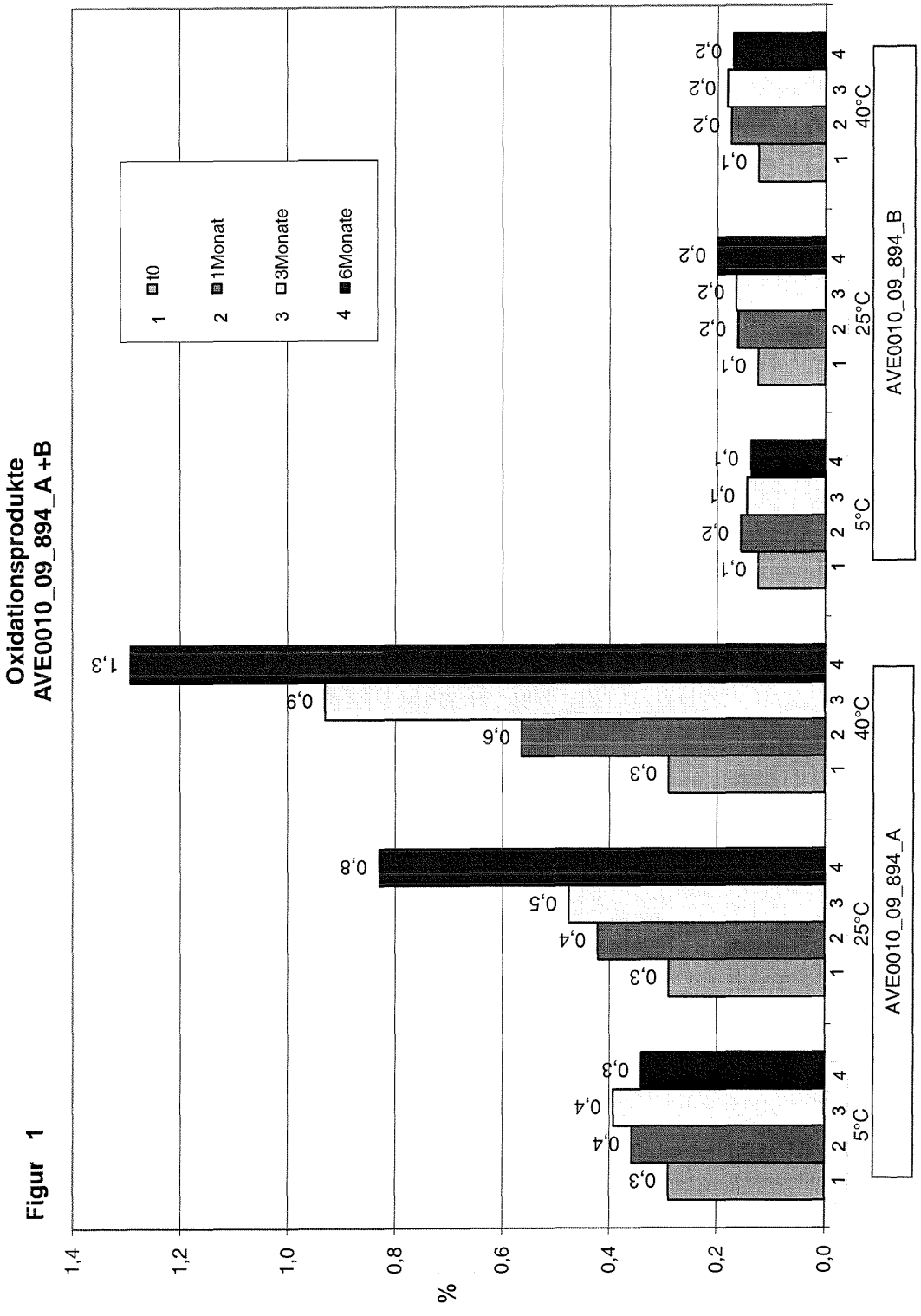
12. Sammensetning for anvendelsen ifølge ett av kravene 7 til 11 for administrering hos pasienter én gang daglig.

13. Sammensetning ifølge ett av kravene 1 til 6 for anvendelse som et tilsetningsstoff til en diett for administrering til pasienter med diabetes type II for å forbedre blodsukkerkontrollen.

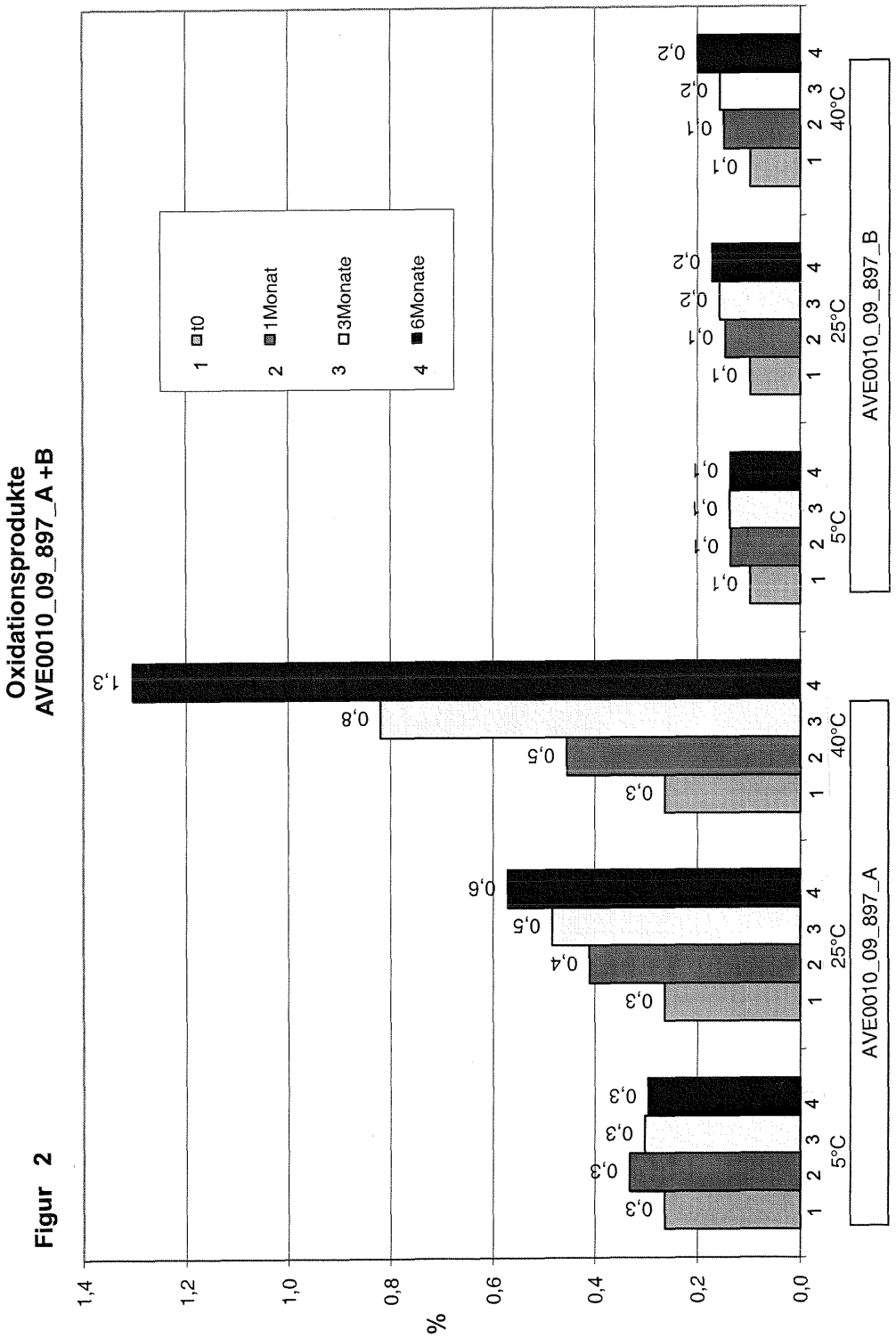
14. Sammensetning for anvendelsen ifølge krav 13 for administrering hos pasienter én gang daglig.

Figurer

Figur 1



Figur 2


Oxidationsprodukte
AVE0010_09_897_A +B
1 t0
2 1Monat
3 3Monate
4 6Monate
%
1,4
1,2
1,0
0,8
0,6
0,4
0,2
0,0
AVE0010_09_897_A
AVE0010_09_897_B
5°C
25°C
40°C
0,3
0,3
0,3
0,3
0,3
0,4
0,5
0,6
0,3
0,5
0,8
1,3
0,1
0,1
0,1
0,1
0,1
0,1
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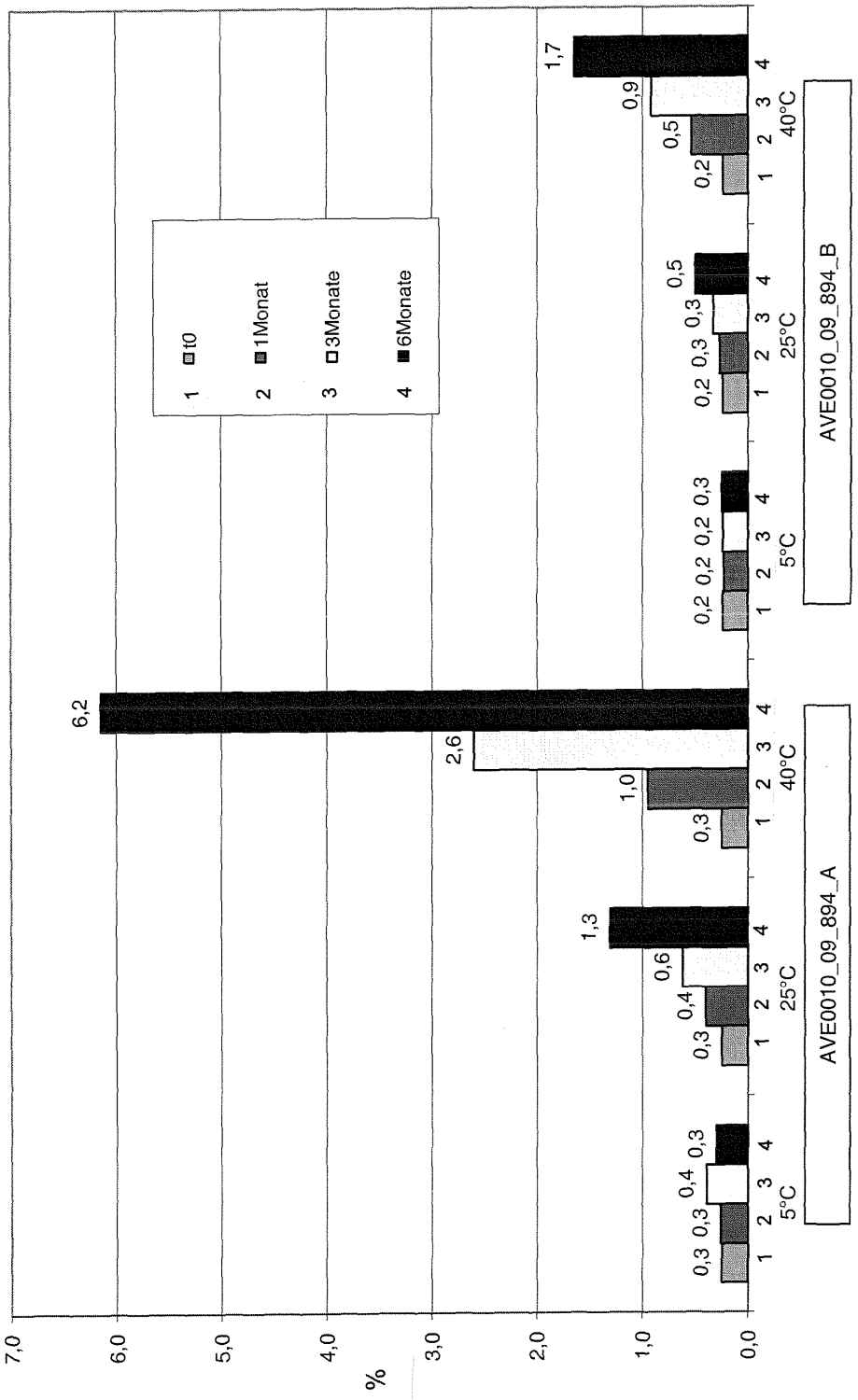
0,2
0,2

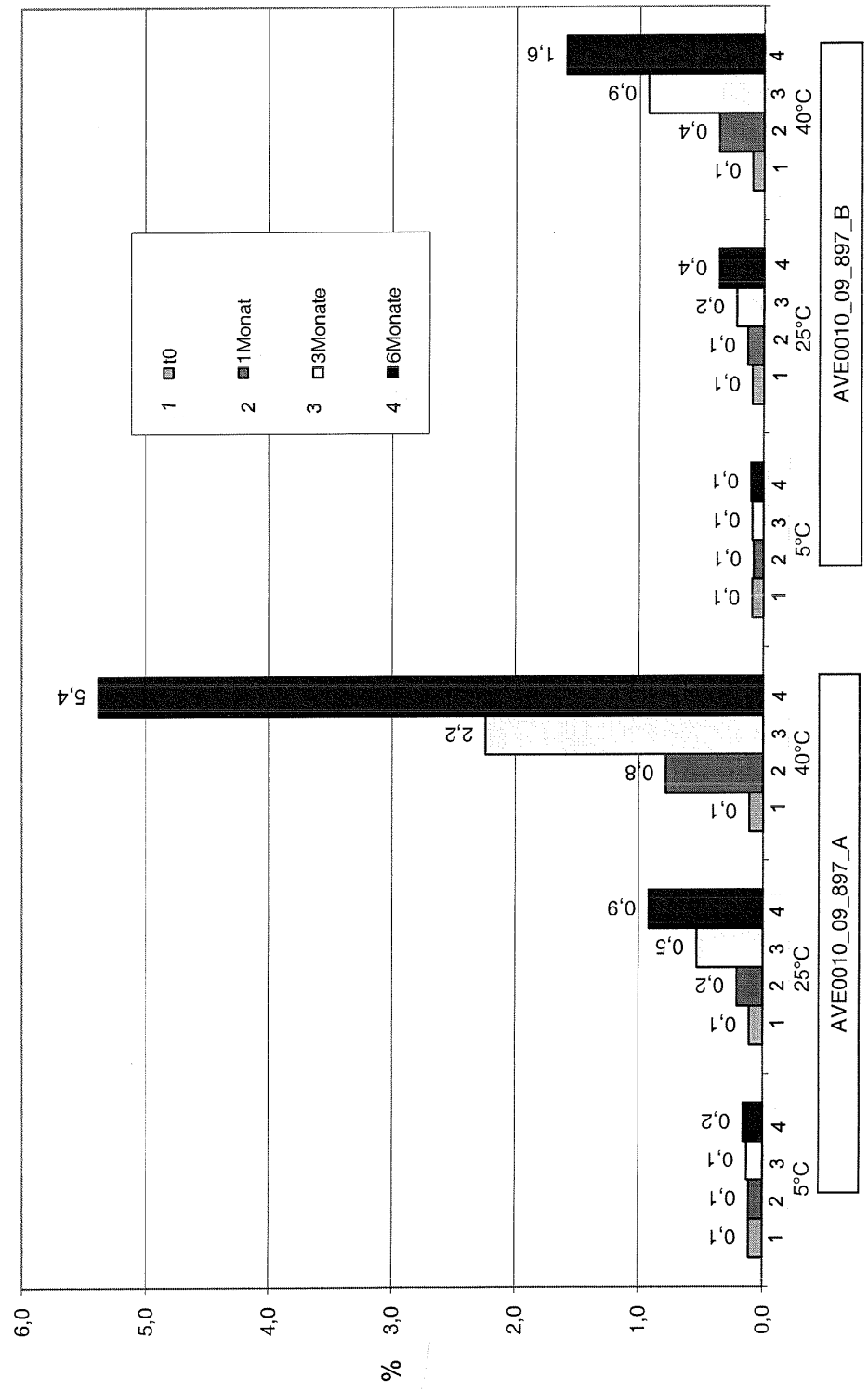
Figur 3

Proteine mit hohem Molekulargewicht
AVE0010_09_894_A + B

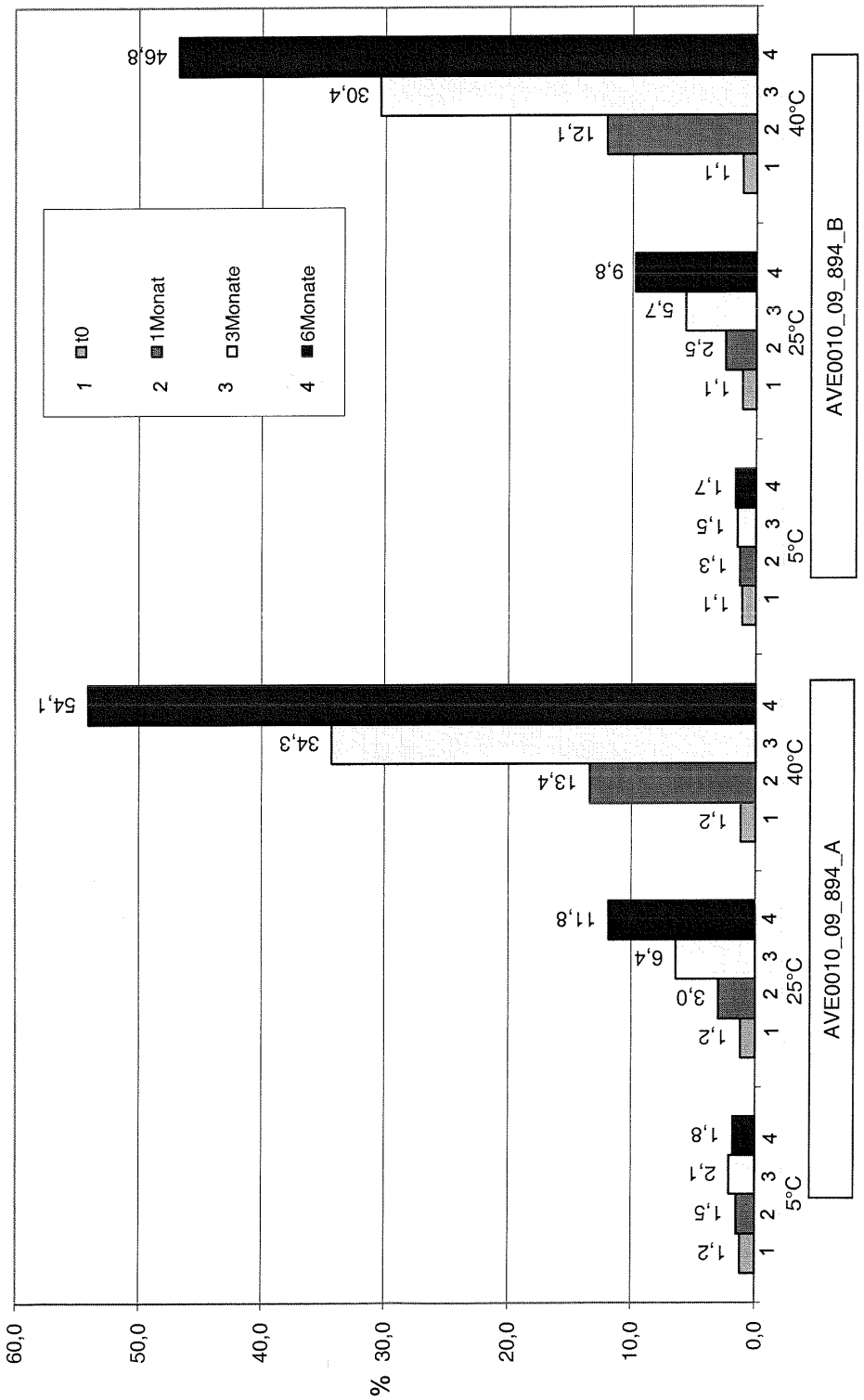


Figur 4

Proteine mit hohem Molekulargewicht
AVE0010_09_897_A + B



Figur 5


Gesamte Verunreinigungen
AVE0010_09_894_A + B
% 60,0
50,0
40,0
30,0
20,0
10,0
0,0
t0
1Monat
3Monate
6Monate
AVE0010_09_894_A
AVE0010_09_894_B
5°C
25°C
40°C
1,2
1,5
2,1
1,8
1,2
3,0
6,4
11,8
1,2
13,4
34,3
54,1
1,1
1,3
1,5
1,7
1,1
2,5
5,7
9,8
1,1
12,1
30,4
46,8

Figur 6

Gesamte Verunreinigungen
AVE0010_09_897_A + B