

**FORMULATIONS OF GLUCAGON-LIKE-PEPTIDE-2 (GLP-2) ANALOGUES AND
THEIR USES**

Field of the Invention

- 5 The present invention relates to formulations of glucagon-like-peptide-2 (GLP-2) analogues and their medical uses, in particular in the treatment and/or prevention of stomach and bowel-related disorders and for ameliorating side effects of chemotherapy and radiation therapy.

10 **Background of the Invention**

- Human GLP-2 is a 33-amino-acid peptide with the following sequence: Hy-His-Ala-Asp-Gly-Ser-Phe-Ser-Asp-Glu-Met-Asn-Thr-Ile-Leu-Asp-Asn-Leu-Ala-Ala-Arg-Asp-Phe-Ile-Asn-Trp-Leu-Ile-Gln-Thr-Lys-Ile-Thr-Asp-OH (SEQ ID NO: 11). It is derived from specific post-translational processing of proglucagon in the enteroendocrine L cells of the intestine and in specific regions of the brainstem. GLP-2 binds to a single G-protein-coupled receptor belonging to the class II glucagon secretin family.

- GLP-2 has been reported to induce significant growth of the small intestinal mucosal epithelium via the stimulation of stem cell proliferation in the crypts, and by inhibition of apoptosis in the villi (Drucker et al., 1996, Proc. Natl. Acad. Sci. USA 93: 7911-7916). GLP-2 also has growth effects on the colon. Furthermore, GLP-2 inhibits gastric emptying and gastric acid secretion (Wojdemann et al., 1999, J. Clin. Endocrinol. Metab. 84: 2513-2517), enhances intestinal barrier function (Benjamin et al., 2000, Gut 47: 112-119), stimulates intestinal hexose transport via the upregulation of glucose transporters (Cheeseman, 1997, Am. J. Physiol. R1965-71), and increases intestinal blood flow (Guan et al., 2003, Gastroenterology, 125: 136-147).

- It has been recognised in the art that glucagon-like peptide-2 receptor analogues have therapeutic potential for the treatment of intestinal diseases. However, the native hGLP-2, a 33 amino acid gastrointestinal peptide, is not a useful in a clinical setting due to its very short half-life in humans of around 7 minutes for full length GLP-2 [1-33] and 27 minutes for truncated GLP-2 [3-33]. In large part, the short half-life is due to degradation by the enzyme dipeptidylpeptidase IV (DPP-IV). Accordingly, there have been attempts in the art to develop GLP-2 receptor agonists with better pharmacokinetic characteristics, in particular to improve the half-life of GLP-2 molecules. By way of example, GLP-2 analogues with substitutions have been suggested such as e.g. GLP-2 analogues containing Gly substitution at position 2 ([hGly2] GLP-2, teduglutide) which increases the

half-life from seven minutes (native GLP-2) to about two hours. Teduglutide is approved for the treatment of short bowel syndrome under the name Gattex (in the United States) and Revestive (in Europe).

- 5 WO 2006/117565 (Zealand Pharma A/S) describes GLP-2 analogues which comprise one of more substitutions as compared to [hGly2]GLP-2 and which improved biological activity in vivo and/or improved chemical stability, e.g. as assessed in in vitro stability assays. Among the molecules disclosed in WO 2006/117565 are ZP1848 (glepaglutide) and ZP1846 (elsiglutide) which have been designed to be stable in liquid formulations.
- 10 Dosage regimes for GLP-2 analogues including ZP1848 and its metabolites are described in WO 2018/229252, which also shows that these compounds are effective to increase longitudinal growth of the intestines. Ready-to-use formulations of ZP1848 and ZP1846 are described in WO 2020/065064, and in particular that the viscosity of the formulations is dependent on the concentration of acetate present and that the
- 15 concentration of covalently bound oligomers is inversely dependent on the increasing concentration of the GLP-2 analogue.

It remains a problem in this area to improve the performance of formulations of GLP-2 analogues, in particular to provide stable liquid formulations that are capable of long term

20 storage without unacceptable levels of physical or chemical degradation of the active monomeric form of the peptide occurring. This is because the shelf life of liquid formulations of GLP-2 analogues, such as glepaglutide (ZP1848) and elsiglutide (ZP1846), is limited by physical and/or chemical degradation decreasing the drug content of the formulations. For peptide therapeutics, regulatory authorities typically have

25 standards that limit the extent of degradation products that are permitted in formulations following distribution and storage, for example to assure a minimum peptide drug content of 90% of the labelled content.

Accordingly, there is a challenge in the field of therapeutic polypeptides and antibodies to

30 maintain the activity of the drug substance, especially when it is intended for long term storage in the form of aqueous liquid formulations, and particularly when the polypeptide or antibody contains amino acid residues that are prone to undergoing degradation reactions, such as methionine, asparagine, aspartic acid and lysine, in degradation reactions such as asparagine deamidation, aspartate isomerization, methionine

35 oxidation, and lysine glycation.

Summary of the Invention

Broadly, the present invention is based on experiments in which liquid aqueous formulations or liquid drug products of glepaglutide (ZP1848) were stored under accelerated conditions to determine the degradation product profile of the GLP-2 analogue in the formulation and hence to determine the suitability of the formulations for long term storage. In the course of these investigations, the present inventors found that a number of possible mechanisms were responsible for the degradation of ZP1848, leading to a range of different degradation products. In particular, in the course of UHPLC and LC-MS testing, they found that one of the main degradation products that resulted from the isomerisation of the Asp (D) residue at position 33 of ZP1848 to form an Iso-Asp/Beta Asp residue, referred to herein as "isoAsp(33)" or "isoAsp(33) ZP1848", was responsible for approximately half of the loss of content of ZP1848 in the formulations. The use of UHPLC enabled the detection of the isoAsp(33) ZP1848 because although isoAsp(33) has same mass as ZP1848, the degradation product could be identified and detected in the presence of ZP1848 as its UHPLC retention time was different both to ZP1848 (relative retention time 1.15 compared to 1.00 for ZP1848) and to other iso-Asp degradation products such as isoAsp(15) ZP1848 (relative retention time 0.65) and isoAsp(21) ZP1848 (relative retention time 1.19). This unambiguous identification of isoAsp(33) ZP1848 is based on knowledge of potential impurities, testing of synthesized impurities with same mass as ZP1848 and testing of retention time relative to ZP1848.

However, unlike examples of other peptide drug substances or therapeutic monoclonal antibodies where this degradation of aspartate residues by this mechanism occurs, and in contrast to other minor degradation pathways of ZP1848 and ZP1846, the present inventors surprisingly found that the isoAsp(33) degradation product is equally or more potent than the main compound ZP1848 and the metabolite of isoAsp(33) is equally potent to isoAsp(33). IsoAsp(33) is therefore considered as a related substance and contributes to the activity of the pharmacodynamic effects of ZP1848 drug product on the GLP-2 receptor. This surprising observation is associated with isoAsp(33), but not to other isoAsp degradation products of ZP1848, namely isoAsp(15) ZP1848 (ZP2531) and isoAsp(21) (ZP4201), see Table 2 below.

Accordingly, it is justified to add the content of isoAsp(33) ZP1848 to the content of ZP1848 in the calculation of the total content in the ZP1848 drug product. When isoAsp(33) is included in the content, its contribution to the total content increases over time as the content of ZP1848 decreases and the content of isoAsp(33) increases over

time. As shown in the Examples, this has the benefit of extending the shelf life of the formulation with regard to content with at least 5 months to at least 27 months when the drug product is stored in (e.g.) a refrigerator at 2-8°C, because the drop in content is lower and it takes thus longer time for the drug product to reach down to 90% of the
5 labelled content.

Due to the activity of the isoAsp(33) degradation product, inclusion of this degradation product in the calculated content further increases the possibility that the drug product may be stored for a proportion of its shelf life under non-refrigerated conditions, rather
10 than always being stored under refrigerated conditions until use. By way of example, the drug product may be stored at 2 to 8°C for a certain number of months and then be kept for a certain number of days or weeks at a higher (e.g. non-refrigerated) temperature, for example around 20°C, 25°C or 30°C. The storage at room temperature can be extended as desired, but at the expense of storage under refrigerated conditions (2-8°C). This
15 storage under room temperature, which also may be referred to consecutive storage, dual storage, in-use conditions or additional storage conditions, has the advantage that the patients are more flexible when using the drug or drug product, because they can leave the drug outside the refrigerator for a certain number of weeks (when, e.g., travelling) without the risk that the drug product loses activity.

20 Examples of the conventional situation in which the isomerization of Asp residues to isoAsp in a polypeptide or antibody leads to a loss of activity include US Patent Publication No: 2012/0172295 (Indiana University Research and Technology Corp). This describes glucagon peptides with enhanced GIP activity produced by substituting amino
25 acids that are otherwise prone to degradation reactions that would cause a loss of stability, for example by substituting the Asp residue at position 21 of glucagon with Glu. Similarly, Habberger et al. (MAbs, 6(2): 327-339, 2014) review the degradation reactions of a range of recombinantly produced therapeutic monoclonal antibodies and conclude that Asp isomerisation to isoAsp generally leads to a pronounced loss of activity in
30 antibodies, such as trastuzumab.

In accordance with the results in the examples, the GLP-2 analogues used in the formulations and medical uses of the present invention include an aspartic acid residue (Asp) at a position that corresponds to position 33 of native human GLP-2 and include a
35 C-terminal peptide sequence of 1-6 amino acid units of Lys, in the case of ZP1848 Lys₆.

In a first study in the examples, the degradation of ZP1848 to isoAsp(33) ZP1848 was found to be pronounced under all stressed test conditions (higher temperature, and pH), within the four weeks of the study. During storage at 2-8°C for 4 weeks, formation of isoAsp(33) is small or insignificant. The formation of isoAsp(33) in liquid aqueous formulations will become more significant over time at all storage conditions.

A second study in the examples confirmed that isoAsp(33) ZP1848 had significant potency as a related substance to ZP1848 (see Table 2), contributing to the activity of glepaglutide drug product on the GLP-2 receptor. The study also revealed that isoAsp(15) ZP1848 and isoAsp(21) ZP1848 showed little or no potency on the GLP-2 receptor.

A third study was carried out using historical batches of ZP1848 representing the stored commercial ZP1848/glepaglutide drug product. These were tested for stability and found that in a drug product batch comprising ZP1848 and isoAsp(33) ZP1848, isoAsp(33) ZP1848 contributes to the content with an estimated of 1.4% of the content after 12 months, 2.1% of the content after 18 months 2.6% of the content after 24 months, 3.3% of the content after 30 months, and 3.5% of the content after 36 months (absolute values). This in turn means that the shelf life of aqueous formulations of ZP1848/glepaglutide may be extended significantly with regard to content.

A fourth *in vivo* study confirmed that ZP1848 and isoAsp(33) ZP1848 cause significant growth stimulation of the small intestine compared to vehicle treatment (Figure 2).

Accordingly, in a first aspect, the present invention provides a GLP-2 analogue is represented by the formula:

H-HGEGTFSSSELATILDALAARDFIWLIATKIT-[Iso-Asp]-KKKKKK-NH₂ (SEQ ID NO: 4);
H-HGEGTFSSSELATILDALAARDFIWLIATKIT-[Iso-Asp]-KK-OH (SEQ ID NO: 5);
H-HGEGTFSSSELATILDALAARDFIWLIATKIT-[Iso-Asp]-K-OH (SEQ ID NO: 6);
H-HGEGSFSSSELSTILDALAARDFIWLIATKIT-[Iso-Asp]-KKKKKK-NH₂ (SEQ ID NO: 9);
H-HGEGSFSSSELSTILDALAARDFIWLIATKIT-[Iso-Asp]-K-OH (SEQ ID NO: 10)

or a pharmaceutically acceptable salt thereof, for use in therapy.

In a further aspect, the present invention provides a stable liquid pharmaceutical formulation for use in therapy, the formulation comprising as active ingredients a first glucagon-like peptide 2 (GLP-2) analogue, wherein the first GLP-2 analogue is represented by the formula:

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H-HGEGTFSSELATILDALAARDFIAWLIATKIT-[Iso-Asp]-KKKKKK-NH₂ (SEQ ID NO: 4);
or a pharmaceutically acceptable salt thereof; and

a second glucagon-like peptide 2 (GLP-2) analogue, wherein the second GLP-2
10 analogue is represented by the formula:

H-HGEGTFSSELATILDALAARDFIAWLIATKITDKKKKKK-NH₂ (SEQ ID NO: 1);
or a pharmaceutically acceptable salt thereof.

15 In a further aspect, the present invention provides a GLP-2 analogue represented by the formula

H-HGEGTFSSELATILDALAARDFIAWLIATKIT-[Iso-Asp]-KKKKKK-NH₂ (SEQ ID NO: 4);
H-HGEGTFSSELATILDALAARDFIAWLIATKIT-[Iso-Asp]-KK-OH (SEQ ID NO: 5);
20 H-HGEGTFSSELATILDALAARDFIAWLIATKIT-[Iso-Asp]-K-OH (SEQ ID NO: 6);

H-HGEGSFSELSTILDALAARDFIAWLIATKIT-[Iso-Asp]-KKKKKK-NH₂ (SEQ ID NO: 9);
H-HGEGSFSELSTILDALAARDFIAWLIATKIT-[Iso-Asp]-K-OH (SEQ ID NO: 10);

25 or a pharmaceutically acceptable salt thereof, for use in a method for the treatment and/or prevention of a stomach and bowel-related disorder in a human patient.

In a further aspect, the present invention provides a stable liquid pharmaceutical formulation for use in therapy, the formulation comprising as an active ingredient a
30 glucagon-like peptide 2 (GLP-2) analogue, wherein the GLP-2 analogue is represented by the formula:

H-HGEGTFSSELATILDALAARDFIAWLIATKIT-[Iso-Asp]-KKKKKK-NH₂ (SEQ ID NO: 4);
H-HGEGTFSSELATILDALAARDFIAWLIATKIT-[Iso-Asp]-KK-OH (SEQ ID NO: 5);
35 H-HGEGTFSSELATILDALAARDFIAWLIATKIT-[Iso-Asp]-K-OH (SEQ ID NO: 6);

H-HGEGSFSELSTILDALAARDFIAWLIATKIT-[Iso-Asp]-KKKKKK-NH₂ (SEQ ID NO: 9);
H-HGEGSFSELSTILDALAARDFIAWLIATKIT-[Iso-Asp]-K-OH (SEQ ID NO: 10);

or a pharmaceutically acceptable salt thereof, wherein the formulation comprises a GLP-2 analogue at a concentration of about 2 mg/mL to about 30 mg/ml, a buffer selected from the group consisting of a histidine buffer, mesylate buffer and acetate buffer, the
5 buffer being present at a concentration of about 5 mM to about 50 mM, a non-ionic tonicity modifier selected from the group consisting of mannitol, sucrose, glycerol and sorbitol at a concentration of about 90 mM to about 360 mM, arginine q.s. to provide a pH of about 6.6 to about 7.4.

10 In a further aspect, the present invention provides a stable liquid pharmaceutical formulation for use in a method for the treatment and/or prevention of a stomach and bowel-related disorder in a human patient, the formulation comprising as an active ingredient a glucagon-like peptide 2 (GLP-2) analogue, wherein the GLP-2 analogue is represented by the formula:

15 H-HGEGTFSSELATILDALAARDFIAWLIATKIT-[Iso-Asp]-KKKKKK-NH₂ (SEQ ID NO: 4);
or
H-HGEGSFSELSTILDALAARDFIAWLIATKIT-[Iso-Asp]-KKKKKK-NH₂ (SEQ ID NO: 9,

20 or a pharmaceutically acceptable salts or metabolites thereof,

wherein the formulation comprises a GLP-2 analogue at a concentration of about 2 mg/mL to about 30 mg/ml, a buffer selected from the group consisting of a histidine buffer, mesylate buffer and acetate buffer, the buffer being present at a concentration of
25 about 5 mM to about 50 mM, a non-ionic tonicity modifier selected from the group consisting of mannitol, sucrose, glycerol and sorbitol at a concentration of about 90 mM to about 360 mM, arginine q.s. to provide a pH of about 6.6 to about 7.4.

In a further aspect, the present invention provides a GLP-2 analogue for use in therapy ,
30 wherein the GLP-2 analogue is represented by the formula:

R¹-Z¹-His-Gly-Glu-Gly-X⁵-Phe-Ser-Ser-Glu-Leu-X¹¹-Thr-Ile-Leu-Asp-Ala-Leu-
Ala-Ala-Arg-Asp-Phe-Ile-Ala-Trp-Leu-Ile-Ala-Thr-Lys-Ile-Thr-[Iso-Asp]-Z²-R²
(SEQ ID NO: 12)

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wherein:

R¹ is hydrogen, C₁₋₄ alkyl (e.g. methyl), acetyl, formyl, benzoyl or trifluoroacetyl;

X5 is Ser or Thr;

X11 is Ala or Ser;

R² is NH₂ or OH; and

Z¹ and Z² are independently absent or a peptide sequence of 1-6 amino acid units of Lys;

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or a pharmaceutically acceptable salt thereof.

In a further aspect, the present invention provides a GLP-2 analogue for use in a method for the treatment and/or prevention of a stomach and bowel-related disorder in a human patient, wherein the GLP-2 analogue is represented by the formula:

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R¹-Z¹-His-Gly-Glu-Gly-X5-Phe-Ser-Ser-Glu-Leu-X11-Thr-Ile-Leu-Asp-Ala-Leu-Ala-Ala-Arg-Asp-Phe-Ile-Ala-Trp-Leu-Ile-Ala-Thr-Lys-Ile-Thr-[Iso-Asp]-Z²-R²
(SEQ ID NO: 12)

15

wherein:

R¹ is hydrogen, C₁₋₄ alkyl (e.g. methyl), acetyl, formyl, benzoyl or trifluoroacetyl;

X5 is Ser or Thr;

X11 is Ala or Ser;

20

R² is NH₂ or OH; and

Z¹ and Z² are independently absent or a peptide sequence of 1-6 amino acid units of Lys;

or a pharmaceutically acceptable salt thereof.

In a further aspect, the present invention provides a stable liquid pharmaceutical formulation for use in therapy, the formulation comprising a glucagon-like peptide 2 (GLP-2) analogue, wherein the GLP-2 analogue is represented by the formula:

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R¹-Z¹-His-Gly-Glu-Gly-X5-Phe-Ser-Ser-Glu-Leu-X11-Thr-Ile-Leu-Asp-Ala-Leu-Ala-Ala-Arg-Asp-Phe-Ile-Ala-Trp-Leu-Ile-Ala-Thr-Lys-Ile-Thr-[Iso-Asp]-Z²-R²
(SEQ ID NO: 12)

wherein:

R¹ is hydrogen, C₁₋₄ alkyl (e.g. methyl), acetyl, formyl, benzoyl or trifluoroacetyl;

35

X5 is Ser or Thr;

X11 is Ala or Ser;

R² is NH₂ or OH; and

Z¹ and Z² are independently absent or a peptide sequence of 1-6 amino acid units of Lys;

or a pharmaceutically acceptable salt thereof;

5 wherein the formulation comprises:

(a) the GLP-2 analogue at a concentration of about 2 mg/mL to about 30 mg/mL;

(b) a buffer selected from the group consisting of a histidine buffer, mesylate buffer,
10 acetate buffer, glycine buffer, lysine buffer, TRIS buffer, Bis-Tris buffer and MOPS buffer,
the buffer being present at a concentration of about 5 mM to about 50 mM;

(c) a non-ionic tonicity modifier selected from the group consisting of mannitol, sucrose,
glycerol, sorbitol and trehalose at a concentration of about 90 mM to about 360 mM; and
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(d) arginine q.s. to provide a formulation having a pH of about 6.6 to about 7.4.

In a further aspect, the present invention provides a stable liquid pharmaceutical
formulation for use in a method for the treatment and/or prevention of a stomach and
20 bowel-related disorder in a human patient, the formulation comprising a glucagon-like
peptide 2 (GLP-2) analogue, wherein the GLP-2 analogue is represented by the formula:

R¹-Z¹-His-Gly-Glu-Gly-X5-Phe-Ser-Ser-Glu-Leu-X11-Thr-Ile-Leu-Asp-Ala-Leu-
Ala-Ala-Arg-Asp-Phe-Ile-Ala-Trp-Leu-Ile-Ala-Thr-Lys-Ile-Thr-[Iso-Asp]-Z²-R²
25 (SEQ ID NO: 12)

wherein:

R¹ is hydrogen, C₁₋₄ alkyl (e.g. methyl), acetyl, formyl, benzoyl or trifluoroacetyl;

X5 is Ser or Thr;

30 X11 is Ala or Ser;

R² is NH₂ or OH; and

Z¹ and Z² are independently absent or a peptide sequence of 1-6 amino acid units of Lys;

or a pharmaceutically acceptable salt thereof;

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wherein the formulation comprises:

- (a) the GLP-2 analogue at a concentration of about 2 mg/mL to about 30 mg/mL;
- (b) a buffer selected from the group consisting of a histidine buffer, mesylate buffer, acetate buffer, glycine buffer, lysine buffer, TRIS buffer, Bis-Tris buffer and MOPS buffer,
 5 the buffer being present at a concentration of about 5 mM to about 50 mM;
- (c) a non-ionic tonicity modifier selected from the group consisting of mannitol, sucrose, glycerol, sorbitol and trehalose at a concentration of about 90 mM to about 360 mM; and
- 10 (d) arginine q.s. to provide a formulation having a pH of about 6.6 to about 7.4.
- In a further aspect, the present invention provides a stable liquid pharmaceutical formulation, the formulation comprising a glucagon-like peptide 2 (GLP-2) analogue, wherein the GLP-2 analogue is represented by the formula:

15 R^1 -Z¹-His-Gly-Glu-Gly-X₅-Phe-Ser-Ser-Glu-Leu-X₁₁-Thr-Ile-Leu-Asp-Ala-Leu-Ala-Ala-Arg-Asp-Phe-Ile-Ala-Trp-Leu-Ile-Ala-Thr-Lys-Ile-Thr-Asp-Z²-R² (SEQ ID NO: 13)

wherein:

- 20 R¹ is hydrogen, C₁₋₄ alkyl (e.g. methyl), acetyl, formyl, benzoyl or trifluoroacetyl;
 X₅ is Ser or Thr;
 X₁₁ is Ala or Ser;
 R² is NH₂ or OH; and
 Z¹ and Z² are independently absent or a peptide sequence of 1-6 amino acid units of Lys;
 25 or a pharmaceutically acceptable salt thereof;

wherein the formulation comprises:

- 30 (a) the GLP-2 analogue at a concentration of about 2 mg/mL to about 30 mg/mL;
- (b) a buffer selected from the group consisting of a histidine buffer, mesylate buffer, acetate buffer, glycine buffer, lysine buffer, TRIS buffer, Bis-Tris buffer and MOPS buffer,
 the buffer being present at a concentration of about 5 mM to about 50 mM;
- 35 (c) a non-ionic tonicity modifier selected from the group consisting of mannitol, sucrose, glycerol, sorbitol and trehalose at a concentration of about 90 mM to about 360 mM; and

(d) arginine q.s. to provide a formulation having a pH of about 6.6 to about 7.4;

wherein the formulation contains at least 1.0% of a GLP-2 analogue represented by the
5 formula:

R¹-Z¹-His-Gly-Glu-Gly-X₅-Phe-Ser-Ser-Glu-Leu-X₁₁-Thr-Ile-Leu-Asp-Ala-Leu-
Ala-Ala-Arg-Asp-Phe-Ile-Ala-Trp-Leu-Ile-Ala-Thr-Lys-Ile-Thr-[Iso-Asp]-Z²-R²
(SEQ ID NO: 12)

10 as a biologically active degradation product.

In a further aspect, the present invention provides a stable liquid pharmaceutical
formulation for use in therapy, the formulation comprising a glucagon-like peptide 2
15 (GLP-2) analogue, wherein the GLP-2 analogue is represented by the formula:

R¹-Z¹-His-Gly-Glu-Gly-X₅-Phe-Ser-Ser-Glu-Leu-X₁₁-Thr-Ile-Leu-Asp-Ala-Leu-
Ala-Ala-Arg-Asp-Phe-Ile-Ala-Trp-Leu-Ile-Ala-Thr-Lys-Ile-Thr-Asp-Z²-R² (SEQ ID
NO: 13)

20 wherein:

R¹ is hydrogen, C₁₋₄ alkyl (e.g. methyl), acetyl, formyl, benzoyl or trifluoroacetyl;

X₅ is Ser or Thr;

X₁₁ is Ala or Ser;

25 R² is NH₂ or OH; and

Z¹ and Z² are independently absent or a peptide sequence of 1-6 amino acid units of Lys;

or a pharmaceutically acceptable salt thereof;

30 wherein the formulation comprises:

(a) the GLP-2 analogue at a concentration of about 2 mg/mL to about 30 mg/mL;

(b) a buffer selected from the group consisting of a histidine buffer, mesylate buffer,
35 acetate buffer, glycine buffer, lysine buffer, TRIS buffer, Bis-Tris buffer and MOPS buffer,
the buffer being present at a concentration of about 5 mM to about 50 mM;

(c) a non-ionic tonicity modifier selected from the group consisting of mannitol, sucrose, glycerol, sorbitol and trehalose at a concentration of about 90 mM to about 360 mM; and

(d) arginine q.s. to provide a formulation having a pH of about 6.6 to about 7.4;

5

wherein the formulation contains at least 1.0% of a GLP-2 analogue represented by the formula:

10 R¹-Z¹-His-Gly-Glu-Gly-X5-Phe-Ser-Ser-Glu-Leu-X11-Thr-Ile-Leu-Asp-Ala-Leu-
Ala-Ala-Arg-Asp-Phe-Ile-Ala-Trp-Leu-Ile-Ala-Thr-Lys-Ile-Thr-[Iso-Asp]-Z²-R²
(SEQ ID NO: 12)

as a biologically active degradation product.

15 In a further aspect, the present invention provides a stable liquid pharmaceutical formulation for use in a method for the treatment and/or prevention of a stomach and bowel-related disorder in a human patient, the formulation comprising a glucagon-like peptide 2 (GLP-2) analogue, wherein the GLP-2 analogue is represented by the formula:

20 R¹-Z¹-His-Gly-Glu-Gly-X5-Phe-Ser-Ser-Glu-Leu-X11-Thr-Ile-Leu-Asp-Ala-Leu-
Ala-Ala-Arg-Asp-Phe-Ile-Ala-Trp-Leu-Ile-Ala-Thr-Lys-Ile-Thr-Asp-Z²-R² (SEQ ID
NO: 13)

wherein:

25 R¹ is hydrogen, C₁₋₄ alkyl (e.g. methyl), acetyl, formyl, benzoyl or trifluoroacetyl;
X5 is Ser or Thr;
X11 is Ala or Ser;
R² is NH₂ or OH; and
Z¹ and Z² are independently absent or a peptide sequence of 1-6 amino acid units of Lys;

30

or a pharmaceutically acceptable salt thereof;

wherein the formulation comprises:

35 (a) the GLP-2 analogue at a concentration of about 2 mg/mL to about 30 mg/mL;

(b) a buffer selected from the group consisting of a histidine buffer, mesylate buffer,

acetate buffer, glycine buffer, lysine buffer, TRIS buffer, Bis-Tris buffer and MOPS buffer, the buffer being present at a concentration of about 5 mM to about 50 mM;

(c) a non-ionic tonicity modifier selected from the group consisting of mannitol, sucrose, glycerol, sorbitol and trehalose at a concentration of about 90 mM to about 360 mM; and

(d) arginine q.s. to provide a formulation having a pH of about 6.6 to about 7.4;

wherein the formulation contains at least 1.0% of a GLP-2 analogue represented by the formula:

R¹-Z¹-His-Gly-Glu-Gly-X5-Phe-Ser-Ser-Glu-Leu-X11-Thr-Ile-Leu-Asp-Ala-Leu-Ala-Ala-Arg-Asp-Phe-Ile-Ala-Trp-Leu-Ile-Ala-Thr-Lys-Ile-Thr-[Iso-Asp]-Z²-R²
(SEQ ID NO: 12)

as a biologically active degradation product.

In a further aspect, the present invention provides a method for the treatment and/or prevention of a stomach and bowel-related disorder in a human patient, the method comprising administering to the human patient a therapeutically effective amount of a GLP-2 analogue represented by the formula:

H-HGEGTFSSSELATILDALAARDFIWLIATKIT-[Iso-Asp]-KKKKKK-NH₂ (SEQ ID NO: 4);
H-HGEGTFSSSELATILDALAARDFIWLIATKIT-[Iso-Asp]-KK-OH (SEQ ID NO: 5);
H-HGEGTFSSSELATILDALAARDFIWLIATKIT-[Iso-Asp]-K-OH (SEQ ID NO: 6);

H-HGEGSFSSSELSTILDALAARDFIWLIATKIT-[Iso-Asp]-KKKKKK-NH₂ (SEQ ID NO: 9);
H-HGEGSFSSSELSTILDALAARDFIWLIATKIT-[Iso-Asp]-K-OH (SEQ ID NO: 10);

or a pharmaceutically acceptable salt thereof.

In a further aspect, the present invention provides a method for the treatment and/or prevention of a stomach and bowel-related disorder in a human patient, the method comprising administering to the human patient a therapeutically effective amount of a formulation comprising as an active ingredient a glucagon-like peptide 2 (GLP-2) analogue, wherein the GLP-2 analogue is represented by the formula:

H-HGEGTFSSSELATILDALAARDFAWLIATKIT-[Iso-Asp]-KKKKKK-NH₂ (SEQ ID NO: 4);

or

H-HGEGSFSSSELSTILDALAARDFAWLIATKIT-[Iso-Asp]-KKKKKK-NH₂ (SEQ ID NO: 9);

- 5 or a pharmaceutically acceptable salts or metabolites thereof, wherein the formulation comprises a GLP-2 analogue at a concentration of about 2 mg/mL to about 30 mg/mL, a buffer selected from the group consisting of a histidine buffer, mesylate buffer and acetate buffer, the buffer being present at a concentration of about 5 mM to about 50 mM, a non-ionic tonicity modifier selected from the group consisting of mannitol, sucrose, 10 glycerol and sorbitol at a concentration of about 90 mM to about 360 mM, arginine q.s. to provide a pH of about 6.6 to about 7.4.

- In a further aspect, the present invention provides a method for the treatment and/or prevention of a stomach and bowel-related disorder in a human patient, the method 15 comprising administering to the human patient a therapeutically effective amount of a formulation comprising as an active ingredient a GLP-2 analogue, wherein the GLP-2 analogue is represented by the formula:

- 20 R¹-Z¹-His-Gly-Glu-Gly-X5-Phe-Ser-Ser-Glu-Leu-X11-Thr-Ile-Leu-Asp-Ala-Leu-Ala-Ala-Arg-Asp-Phe-Ile-Ala-Trp-Leu-Ile-Ala-Thr-Lys-Ile-Thr-[Iso-Asp]-Z²-R²
(SEQ ID NO: 12)

wherein:

R¹ is hydrogen, C₁₋₄ alkyl (e.g. methyl), acetyl, formyl, benzoyl or trifluoroacetyl;

- 25 X5 is Ser or Thr;

X11 is Ala or Ser;

R² is NH₂ or OH; and

Z¹ and Z² are independently absent or a peptide sequence of 1-6 amino acid units of Lys;

- 30 or a pharmaceutically acceptable salt thereof;

wherein the formulation comprises:

- (a) the GLP-2 analogue at a concentration of about 2 mg/mL to about 30 mg/mL;

35

(b) a buffer selected from the group consisting of a histidine buffer, mesylate buffer, acetate buffer, glycine buffer, lysine buffer, TRIS buffer, Bis-Tris buffer and MOPS buffer,

the buffer being present at a concentration of about 5 mM to about 50 mM;

(c) a non-ionic tonicity modifier selected from the group consisting of mannitol, sucrose, glycerol, sorbitol and trehalose at a concentration of about 90 mM to about 360 mM; and

5

(d) arginine q.s. to provide a formulation having a pH of about 6.6 to about 7.4.

In a further aspect, the present invention provides the use of a GLP-2 analogue represented by the formula:

10

H-HGEGTFSSSELATILDALAARDFIAWLIATKIT-[Iso-Asp]-KKKKKK-NH₂ (SEQ ID NO: 4);

H-HGEGTFSSSELATILDALAARDFIAWLIATKIT-[Iso-Asp]-KK-OH (SEQ ID NO: 5);

H-HGEGTFSSSELATILDALAARDFIAWLIATKIT-[Iso-Asp]-K-OH (SEQ ID NO: 6);

15

H-HGEGSFSSSELSTILDALAARDFIAWLIATKIT-[Iso-Asp]-KKKKKK-NH₂ (SEQ ID NO: 9);

H-HGEGSFSSSELSTILDALAARDFIAWLIATKIT-[Iso-Asp]-K-OH (SEQ ID NO: 10);

or a pharmaceutically acceptable salt thereof, in the manufacture of a medicament for the treatment and/or prevention of a stomach and bowel-related disorder in a human patient.

20

In a further aspect, the present invention provides a stable liquid pharmaceutical formulation for use in therapy, the formulation comprising as active ingredients a glucagon-like peptide 2 (GLP-2) analogue, wherein the GLP-2 analogue is represented by the formula:

25

H-HGEGTFSSSELATILDALAARDFIAWLIATKIT-[Iso-Asp]-KKKKKK-NH₂ (SEQ ID NO: 4);

or a pharmaceutically acceptable salt thereof; and

30

a glucagon-like peptide 2 (GLP-2) analogue, wherein the GLP-2 analogue is represented by the formula:

H-HGEGTFSSSELATILDALAARDFIAWLIATKITDKKKKK-NH₂ (SEQ ID NO: 1);

35

or a pharmaceutically acceptable salt thereof.

In a further aspect, the present invention provides the use of a formulation comprising as an active ingredient a glucagon-like peptide 2 (GLP-2) analogue, wherein the GLP-2 analogue is represented by the formula:

- 5 H-HGEGTFSSELATILDALAARDFIAWLIATKIT-[Iso-Asp]-KKKKKK-NH₂ (SEQ ID NO: 4);
or
H-HGEGSFSELSTILDALAARDFIAWLIATKIT-[Iso-Asp]-KKKKKK-NH₂ (SEQ ID NO: 9);

- or a pharmaceutically acceptable salts or metabolites thereof, wherein the formulation
10 comprises a GLP-2 analogue at a concentration of about 2 mg/mL to about 30 mg/mL, a
buffer selected from the group consisting of a histidine buffer, mesylate buffer and
acetate buffer, the buffer being present at a concentration of about 5 mM to about 50
mM, a non-ionic tonicity modifier selected from the group consisting of mannitol, sucrose,
glycerol and sorbitol at a concentration of about 90 mM to about 360 mM, arginine q.s. to
15 provide a pH of about 6.6 to about 7.4 in the manufacture of a medicament for the
treatment and/or prevention of a stomach and bowel-related disorder in a human patient.

- In a further aspect, the present invention provides the use of a formulation comprising as
an active ingredient a GLP-2 analogue, wherein the GLP-2 analogue is represented by
20 the formula:

R¹-Z¹-His-Gly-Glu-Gly-X₅-Phe-Ser-Ser-Glu-Leu-X₁₁-Thr-Ile-Leu-Asp-Ala-Leu-
Ala-Ala-Arg-Asp-Phe-Ile-Ala-Trp-Leu-Ile-Ala-Thr-Lys-Ile-Thr-[Iso-Asp]-Z²-R²
(SEQ ID NO: 12)

25

wherein:

- R¹ is hydrogen, C₁₋₄ alkyl (e.g. methyl), acetyl, formyl, benzoyl or trifluoroacetyl;
X₅ is Ser or Thr;
X₁₁ is Ala or Ser;
30 R² is NH₂ or OH; and
Z¹ and Z² are independently absent or a peptide sequence of 1-6 amino acid units of Lys;

or a pharmaceutically acceptable salt thereof;

- 35 wherein the formulation comprises:

(a) the GLP-2 analogue at a concentration of about 2 mg/mL to about 30 mg/mL;

(b) a buffer selected from the group consisting of a histidine buffer, mesylate buffer, acetate buffer, glycine buffer, lysine buffer, TRIS buffer, Bis-Tris buffer and MOPS buffer, the buffer being present at a concentration of about 5 mM to about 50 mM;

5

(c) a non-ionic tonicity modifier selected from the group consisting of mannitol, sucrose, glycerol, sorbitol and trehalose at a concentration of about 90 mM to about 360 mM; and

(d) arginine q.s. to provide a formulation having a pH of about 6.6 to about 7.4;

10

in the manufacture of a medicament for the treatment and/or prevention of a stomach and bowel-related disorder in a human patient.

In a further aspect, the present invention provides a delivery device containing the GLP-2 analogue or formulation as defined herein, optionally wherein the delivery device is pre-filled syringe, an injector device, an injector pen, an adjustable dose auto-injector, a disposable auto-injector, a wearable injector, an infusion pump.

15

In a further aspect, the present invention provides a method of assaying a formulation comprising a glucagon-like peptide 2 (GLP-2) analogue to determine a total drug substance content of the formulation, wherein the GLP-2 analogue is represented by the formula:

20

R¹-Z¹-His-Gly-Glu-Gly-X⁵-Phe-Ser-Ser-Glu-Leu-X¹¹-Thr-Ile-Leu-Asp-Ala-Leu-
Ala-Ala-Arg-Asp-Phe-Ile-Ala-Trp-Leu-Ile-Ala-Thr-Lys-Ile-Thr-Asp-Z²-R² (SEQ ID
NO: 13)

25

wherein:

R¹ is hydrogen, C₁₋₄ alkyl (e.g. methyl), acetyl, formyl, benzoyl or trifluoroacetyl;

30

X⁵ is Ser or Thr;

X¹¹ is Ala or Ser;

R² is NH₂ or OH; and

Z¹ and Z² are independently absent or a peptide sequence of 1-6 amino acid units of Lys;

35

or a pharmaceutically acceptable salt thereof; wherein the method comprises:

determining an amount of the GLP-2 analogue present in the formulation;

determining an amount of a corresponding isoAsp33 degradation product of the GLP-2 analogue represented by the formula:

5 R¹-Z¹-His-Gly-Glu-Gly-X5-Phe-Ser-Ser-Glu-Leu-X11-Thr-Ile-Leu-Asp-Ala-Leu-
Ala-Ala-Arg-Asp-Phe-Ile-Ala-Trp-Leu-Ile-Ala-Thr-Lys-Ile-Thr-[Iso-Asp]-Z²-R²
(SEQ ID NO: 12)

and adding the amounts determined in steps (a) and (b) to provide the total substance
10 content of the GLP-2 analogue present in the formulation.

The components of the formulation and their amounts provide a formulation with at least
90% content of the GLP-2 analogue and with less than 10% of chemical degradation
products at storage for at least 18 months at 2-8°C.

15 Embodiments of the present invention will now be described by way of example and not
limitation. However, various further aspects and embodiments of the present invention
will be apparent to those skilled in the art in view of the present disclosure. In particular,
where the present invention is described with reference to EPC2000 purpose limited
20 second medical use claims, it should be understood to extent to other applicable claim
formats, such as methods of medical treatment, Swiss format medical use claims and
purpose limited product claims.

“and/or” where used herein is to be taken as specific disclosure of each of the two
25 specified features or components with or without the other. For example “A and/or B” is
to be taken as specific disclosure of each of (i) A, (ii) B and (iii) A and B, just as if each is
set out individually herein.

Unless context dictates otherwise, the descriptions and definitions of the features set out
30 above are not limited to any particular aspect or embodiment of the invention and apply
equally to all aspects and embodiments which are described.

Embodiments of the present invention will now be described by way of example and not
limitation with reference to the accompanying figures. However, various further aspects
35 and embodiments of the present invention will be apparent to those skilled in the art in
view of the present disclosure.

Brief Description of the Figures

Figure 1 shows the decrease in content of ZP1848/glepaglutide drug product when stored at 2-8°C. Content presented as content of ZP1848/glepaglutide and content of ZP1848/glepaglutide plus related substance isoAsp(33) ZP1848.

5

Figure 2 shows growth of small intestinal mass of compound ZP848 and ZP1848 isoAsp(33) compared to vehicle control. Values are means $\pm$ SD. One way ANOVA, 95% confidence interval. Šidák's multiple comparison test, ****= $p < 0.0001$, ns= not significant.

10 **Figure 3** shows the MS spectra from LC-MS analysis of the peak with RRT 1.15 (full view).

Figure 4 shows the MS spectra from LC-MS analysis of the peak with RRT 1.15 (zoomed).

15

Figure 5 shows a UHPLC chromatogram of aged ZP1848/glepaglutide formulation and the degradation peak with relative retention time 1.15.

20 **Figure 6** shows HPLC chromatograms of impurities including iso-Asp(33) spiked into a ZP1848/glepaglutide formulation (upper) and individual iso-Asp(33) injected into UHPLC(lower).

Detailed Description of the Invention**Definitions**

25 Unless specified otherwise, the following definitions are provided for specific terms, which are used in the above written description.

Throughout the description and claims the conventional one-letter and three-letter codes for natural amino acids are used. All amino acid residues in peptides of the invention are preferably of the L-configuration. However, D-configuration amino acids may also be present, notably through the isomerisation of the Asp residue to form Iso-Asp/Beta Asp at position 33 of lepaglutide.

35 Compounds of the present invention and some of their degradation products such as Iso-Asp at position 33 have at least one GLP-2 biological activity, in particular in causing growth of the intestine. This can be assessed in *in vivo* assays, for example as described in the examples in this application or those provided in WO 2006/117565, in

which the mass of the intestine, or a portion thereof, is determined after a test animal has been treated with or exposed to a GLP-2 analogue.

Compounds

- 5 The GLP-2 analogues present in the drug products of the present invention have one or more amino acid substitutions, deletions, inversions, or additions compared with native GLP-2 as explained further below. In accordance with the results in the examples, the GLP-2 analogues used alone or in combination in the formulations and medical uses of the present invention may include an aspartic acid residue (Asp) at a position that
- 10 corresponds to position 33 of native human GLP-2. While not wishing to be bound by any particular theory, the present inventors have found that isomerisation of this residue in ZP1848 or ZP1846 to form an iso-Asp/beta-Asp residue at position 33 provides a degradation product that surprisingly retains GLP-2 biological activity. Accordingly, these results will also apply equally to other GLP-2 analogues with a corresponding Asp
- 15 residue at position 33 in situations in which this residue isomerises to form a corresponding degradation product with an iso-Asp/beta-Asp residue at position 33, in particular GLP-2 analogues such as glepaglutide (ZP1848) and elsiglutide (ZP1846) that have 1-6 C-terminal lysine residues. Alternatively or additionally, the present inventors believe that GLP-2 analogues having a C-terminal lysine tail facilitates the isomerisation
- 20 reaction that creates an isoAsp in position 33, and so GLP-2 analogues with a lysine tail (e.g. in which Z² is present) are preferred embodiments of the present invention.

By way of example, the liquid formulations of the present invention may employ a glucagon-like peptide 2 (GLP-2) analogue represented by the formula:

- 25
- $$\text{R}^1\text{-Z}^1\text{-His-Gly-Glu-Gly-X5-Phe-Ser-Ser-Glu-Leu-X11-Thr-Ile-Leu-Asp-Ala-Leu-Ala-Ala-Arg-Asp-Phe-Ile-Ala-Trp-Leu-Ile-Ala-Thr-Lys-Ile-Thr-Asp-Z}^2\text{-R}^2 \text{ (SEQ ID NO: 1)}$$

- 30 wherein

R¹ is hydrogen, C₁₋₄ alkyl (e.g. methyl), acetyl, formyl, benzoyl or trifluoroacetyl;

X5 is Ser or Thr;

X11 is Ala or Ser;

R² is NH₂ or OH; and

- 35 Z¹ and Z² are independently absent or a peptide sequence of 1-6 amino acid units of Lys;

or a pharmaceutically acceptable salt thereof.

In this case, the degradation product resulting from isomerisation at position Asp 33 is a glucagon-like peptide 2 (GLP-2) analogue represented by the formula:

5 R¹-Z¹-His-Gly-Glu-Gly-X5-Phe-Ser-Ser-Glu-Leu-X11-Thr-Ile-Leu-Asp-Ala-Leu-
Ala-Ala-Arg-Asp-Phe-Ile-Ala-Trp-Leu-Ile-Ala-Thr-Lys-Ile-Thr-[Iso-Asp]-Z²-R²
(SEQ ID NO: 12)

wherein

- R¹ is hydrogen, C₁₋₄ alkyl (e.g. methyl), acetyl, formyl, benzoyl or trifluoroacetyl;
10 X5 is Ser or Thr;
X11 is Ala or Ser;
R² is NH₂ or OH; and
Z¹ and Z² are independently absent or a peptide sequence of 1-6 amino acid units of Lys;
15 or a pharmaceutically acceptable salt thereof.

In some embodiments of the present invention, in the above formula, X5 is Thr and/or X11 is Ala. Examples of these glucagon-like peptide 2 (GLP-2) analogues include the following molecules, with the N-terminal "H" representing hydrogen (i.e. at the position
20 equivalent to R¹ in the general formula above), with the C-terminal group being a hydroxyl or amino group (i.e. at the position equivalent to R² in the general formula above):

ZP1848 H-HGEGTFSSELATILDALAARDFIWLIATKITDKKKKKK-NH₂ (SEQ ID NO: 1);
25 ZP2711 H-HGEGTFSSELATILDALAARDFIWLIATKITDKK-OH (SEQ ID NO: 2);
ZP2469 H-HGEGTFSSELATILDALAARDFIWLIATKITDK-OH (SEQ ID NO: 3);

In this cases, the degradation products that may result from isomerisation at position Asp 33 are:

30 ZP6885 H-HGEGTFSSELATILDALAARDFIWLIATKIT-[Iso-Asp]-KKKKKK-NH₂ (SEQ ID NO: 4)
ZP15294 H-HGEGTFSSELATILDALAARDFIWLIATKIT-[Iso-Asp]-KK-OH (SEQ ID NO: 5);
35 ZP11896 H-HGEGTFSSELATILDALAARDFIWLIATKIT-[Iso-Asp]-K-OH (SEQ ID NO: 6);

In an embodiment of the present invention, the glucagon-like peptide 2 (GLP-2) analogue is ZP1848 H-HGEGTFSSELATILDALAARDFIWLIATKITDKKKKKK-NH₂ (SEQ ID NO: 1).

5 That is, the GLP-2 analogue is represented by the formula:

H-His¹-Gly²-Glu³-Gly⁴-Thr⁵-Phe⁶-Ser⁷-Ser⁸-Glu⁹-Leu¹⁰-Ala¹¹-Thr¹²-Ile¹³-Leu¹⁴-Asp¹⁵-Ala¹⁶-
Leu¹⁷-Ala¹⁸-Ala¹⁹-Arg²⁰-Asp²¹-Phe²²-Ile²³-Ala²⁴-Trp²⁵-Leu²⁶-Ile²⁷-Ala²⁸-Thr²⁹-Lys³⁰-Ile³¹-
Thr³²-Asp³³-Lys³⁴-Lys³⁵-Lys³⁶-Lys³⁷-Lys³⁸-Lys³⁹-NH₂.

10

It will be understood that the N-terminal "H-" indicates a free N-terminal amine (NH₂-) group. The C-terminal "NH₂-" indicates a C-terminal amide group. The terms ZP1848 and glepaglutide may be used interchangeably.

15 In some embodiments of the present invention, in the above formula X5 is Ser and/or X11 is Ser. Examples of these glucagon-like peptide 2 (GLP-2) analogues include:

ZP1846 H-HGEGSFSELSTILDALAARDFIWLIATKITDKKKKKK-NH₂ (SEQ ID NO: 7);
or

20 ZP2242 H-HGEGSFSELSTILDALAARDFIWLIATKITDK-OH (SEQ ID NO: 8).

In this case, the degradation products resulting from isomerisation at position Asp 33 are:

H-HGEGSFSELSTILDALAARDFIWLIATKIT-[Iso-Asp]-KKKKKK-NH₂ (SEQ ID NO: 9);

25 H-HGEGSFSELSTILDALAARDFIWLIATKIT-[Iso-Asp]-K-OH (SEQ ID NO: 10).

In an embodiment of the present invention, the glucagon-like peptide 2 (GLP-2) analogue is ZP1846 H-HGEGSFSELSTILDALAARDFIWLIATKITDKKKKKK-NH₂ (SEQ ID NO: 7).

30

That is, the GLP-2 analogue is represented by the formula:

H-His¹-Gly²-Glu³-Gly⁴-Ser⁵-Phe⁶-Ser⁷-Ser⁸-Glu⁹-Leu¹⁰-Ser¹¹-Thr¹²-Ile¹³-Leu¹⁴-Asp¹⁵-Ala¹⁶-
Leu¹⁷-Ala¹⁸-Ala¹⁹-Arg²⁰-Asp²¹-Phe²²-Ile²³-Ala²⁴-Trp²⁵-Leu²⁶-Ile²⁷-Ala²⁸-Thr²⁹-Lys³⁰-Ile³¹-
35 Thr³²-Asp³³-Lys³⁴-Lys³⁵-Lys³⁶-Lys³⁷-Lys³⁸-Lys³⁹-NH₂.

It will be understood that the N-terminal "H-" indicates a free N-terminal amine (NH₂-) group. The C-terminal "NH₂-" indicates a C-terminal amide group. The terms ZP1846 and elsiglutide may be used interchangeably.

- 5 It should be understood that the peptides (drug substance) of the invention might also be provided in the form of a salt or other derivative. Salts include pharmaceutically acceptable salts, such as acid addition salts and basic salts. Examples of acid addition salts include hydrochloride salts, citrate salts, chloride salts and acetate salts. Preferably, the salt is acetate. In general, it is preferred that the salt is not a chloride salt.
- 10 Examples of basic salts include salts where the cation is selected from alkali metals, such as sodium and potassium, alkaline earth metals, such as calcium, and ammonium ions $^+N(R^3)_3(R^4)$, where R³ and R⁴ independently designates optionally substituted C₁₋₆-alkyl, optionally substituted C₂₋₆-alkenyl, optionally substituted aryl, or optionally substituted heteroaryl. Other examples of pharmaceutically acceptable salts are
- 15 described in "Remington's Pharmaceutical Sciences", 17th edition. Ed. Alfonso R. Gennaro (Ed.), Mark Publishing Company, Easton, PA, U.S.A., 1985 and more recent editions, and in the Encyclopaedia of Pharmaceutical Technology.

ZP1848 is a peptide having the formula:

20



- as described e.g. in WO 2006/117565. It will be understood that the N-terminal "H-" indicates a free N-terminal amine (NH₂) group. The C-terminal "NH₂-" indicates a C-
- 25 terminal amide group. The terms "ZP1848" and "glepaglutide" may be used interchangeably.

- In some cases, the present invention relates to the use of pharmaceutically acceptable salts of ZP1848, as described in more detail below. Any suitable salt may be used,
- 30 although acetate may be preferred.

- When ZP1848 is injected into the subcutaneous (SC) compartment, two functionally active metabolites are formed, ZP2469 and ZP2711, both C-terminal truncated analogues of ZP1848. The overall PK profile of ZP1848 therefore comprises the effect of
- 35 ZP1848 and its two main metabolites. Similarly, ZP1846 forms the C-terminal truncated analogue, the metabolite ZP2242 (SEQ ID NO: 8).

ZP2469 is a peptide having the formula:



5 ZP2711 is a peptide having the formula:



where the N-terminal "H-" is as described above, and the C-terminal "-OH" indicates a
10 free C-terminal carboxylic acid group.

In preferred embodiments, the acetate salt of a GLP-2 analogue of the invention is selected from the group consisting of ZP1848-acetate, ZP2949-acetate, ZP2711-acetate, ZP2469-acetate, ZP1857-acetate, ZP2530-acetate, ZP1846-acetate, ZP1855-acetate
15 and ZP2242-acetate. In the present context, the term "ZP1848-acetate" refers to the ZP1848 molecule is in the form of an acetate salt. The acetate salts of GLP-2 analogues may be represented by the formula (GLP-2 analogue), $x(\text{CH}_3\text{COOH})$ where x is 1.0 to 8.0, i.e. where x is 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0 or 8.0. In any composition of the acetate salts of the GLP-2 analogues, there may be molecules with different number of
20 acetate molecules so that x is not necessarily a whole integer. In some cases, x is from 4.0 to 8.0, x is from 6.0 to 8.0, or x is from 4.0 to 6.5. In some cases, x is from 2.0 to 8.0, 3.0 to 8.0, 4.0 to 8.0, 2.0 to 7.0, 3.0 to 7.0, 4.0 to 7.0, 2.0 to 6.0, 3.0 to 6.0, or 4.0 to 6.0. In one particular embodiment, x to from 2.0 to 6.0.

25 In a preferred embodiment, the GLP-2 analogue is ZP1848-acetate or H-HGEGTFSSELATILDALAARDFIWLIATKITDKKKKKK-NH₂ acetate (SEQ ID NO: 1) or (H-HGEGTFSSELATILDALAARDFIWLIATKITDKKKKKK-NH₂), $x(\text{CH}_3\text{COOH})$ where x is 1.0 to 8.0.

30 Accordingly, in a further aspect, the present invention provides solid compositions comprising an acetate salt of a glucagon-like peptide 2 (GLP-2) analogue. The solid compositions are useful for formulating with the excipients used to make the liquid formulations of the present invention. In one embodiment, the present invention provides a solid composition comprising an acetate salt of a glucagon-like peptide 2 (GLP-2)
35 analogue having the formula:



is 1.0 to 8.0.

An upper limit of 8.0 acetate molecules per GLP-2 analogue equates to an acetate content of less than 11% acetate and may be formulated to have a viscosity between 0.8 and 2.0 mPa/sec measured at 25°C.

The range of the number of acetate molecules associated with each molecule of the GLP-2 analogues defines a molecular weight range for this component of the formulation. For example, for the acetate salts of ZP1848, the range of the number of acetate molecules associated with each molecule of the GLP-2 analogues defines a molecular weight range of the ZP1848-acetate. By way of example, 1 acetate equivalent with each molecule of ZP1848 provides a molecular weight = 4316 + 60 = 4376 Da. Accordingly, the molecular weights for increasing acetate equivalents with ZP1848 are as follows: 1 acetate equivalent = 4376 Da; 2 acetate equivalents = 4436 Da; 3 acetate equivalents = 4496 Da; 4 acetate equivalents = 4556 Da; 5 acetate equivalents = 4616 Da; 6 acetate equivalents = 4676 Da; 7 acetate equivalents = 4736 Da and 8 acetate equivalents = 4796 Da. This in turn defines molecular weight ranges as follows: 1-8 acetate equivalents = 4376 Da - 4796 Da; 4-8 acetate equivalents = 4556 Da - 4796 Da and 6-8 acetate equivalents = 4676 Da - 4796 Da.

Z¹ and Z² are independently present and/or absent or a peptide sequence of 1-6 amino acid units of Lys, i.e. 1, 2, 3, 4, 5 or 6 Lys residues. The Lys residues may have either D- or L-configuration, but have an L-configuration. Particularly preferred sequences Z are sequences of four, five or six consecutive lysine residues, and particularly six consecutive lysine residues. Exemplary sequences Z are shown in WO 01/04156. In certain embodiments, Z¹ is absent. In such cases, Z² may be either present or absent.

Formulations of the GLP-2 analogues

The GLP-2 analogues as used herein may be formulated as pharmaceutical compositions prepared for storage or administration, and which comprise a therapeutically effective amount of the GLP-2 analogue in a pharmaceutically acceptable carrier.

Suitable salts include acid addition salts and basic salts. Examples of acid addition salts include hydrochloride salts, citrate salts, chloride salts and acetate salts. Preferably, the salt is acetate. In general, it is preferred that the salt is not a chloride salt. Examples of basic salts include salts where the cation is selected from alkali metals, such as sodium

and potassium, alkaline earth metals, such as calcium, and ammonium ions +N (R3) 3(R4), where R3 and R4 independently designates optionally substituted C1-6-alkyl, optionally substituted C2-6-alkenyl, optionally substituted aryl, or optionally substituted heteroaryl.

5

Acetate salts may be particularly preferred. In the present context, the term "ZP1848-acetate" refers to the ZP1848 molecule in the form of an acetate salt. The acetate salts of ZP1848 may be represented by the formula (ZP1848), $x(\text{CH}_3\text{COOH})$ where x is 1.0 to 8.0, i.e. where x is 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0 or 8.0. In any composition, there may
10 be molecules with different number of acetate molecules so that x is not necessarily a whole integer. In some cases, x is from 4.0 to 8.0, x is from 6.0 to 8.0, or x is from 4.0 to 6.5. In some cases is from x is from 4.0 to 6.0, x is from 2.0 to 7.0, x is from 2.0 to 6.0, x is from 3.0 to 6.0, x is from 4.0 to 6.0 or x is 4.0 to 8.0.

15 When administration is to be parenteral, such as subcutaneous or intramuscular, injectable pharmaceutical compositions can be prepared in conventional forms. Glepaglutide is generally provided as aqueous liquid formulations, for example as described in WO 2020/065064 and WO 2020/065063, the contents of which are incorporated by reference in their entirety. Subcutaneous administration may be
20 particularly preferred, e.g. by injection.

A human dose (total dose) of ZP1848 may be from about such as between and including 0.1 mg and 25 mg per patient between and including 0.5 mg and 20 mg per patient, such as between and including 1 mg and 15 mg per patient, such as between and including 1
25 mg and 10 mg per patient once or twice weekly, such as between and including 2 mg and 10 mg per patient, or as a plurality of doses as defined herein separated in time by 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 or 14 days. In some instances, a fixed dose of ZP1848 may be used in accordance with a dosing pattern disclosed herein, i.e. a dose which is the same regardless of the body weight of the patient, given once or twice
30 weekly. By way of example, the fixed dose may be a dose of 1 mg, 2 mg, 4 mg, 5 mg, 6 mg, 7 mg, 8 mg, 9 mg, 10 mg, 11 mg, 12 mg, 13 mg, 14 mg or 15 mg. Conveniently, a fixed dose of 10 mg may be used. The use of fixed dosing has the advantage of increasing compliance and reducing the risk of patient dosing errors, including risks of miscalculating a weight based dose to be administered.

35

In preferred embodiments, the formulation is a ready-to-use formulation as described in WO 2020/065064. The term "ready-to-use" as used herein refers to a formulation that

does not require constitution or dilution with a prescribed amount of diluent, e.g., water for injection or other suitable diluent, before use by the designated route of administration.

- 5 In the present invention, the terms "formulation" and "drug product" are used interchangeably.

As described herein, the liquid formulations of the GLP-2 analogues of the present invention include a buffer, a non-ionic tonicity modifier and arginine q.s. to provide the pH
10 of the final formulation. In accordance with normal pharmaceutical practice, the formulations of the present invention are sterile and/or free from reducing agent. In preferred cases, the liquid formulations of the present invention are aqueous, liquid formulations. In some cases, the liquid formulations of the present invention are non-
aqueous, liquid formulations.

15 The term "buffer" as used herein denotes a pharmaceutically acceptable excipient which stabilizes the pH of a pharmaceutical formulation. Suitable buffers are well known in the art and can be found in the literature such as e.g. a histidine buffer, mesylate buffer, acetate buffer, glycine buffer, lysine buffer, TRIS buffer, Bis-Tris buffer or MOPS buffer.
20 In preferred embodiments, the buffer is a histidine buffer, e.g. L-histidine. Generally, the buffer will be present at a concentration of about 5 mM to about 50 mM, more preferably at a concentration of about 5 mM to about 25 mM, and most preferably at a concentration of about 15 mM.

25 The term "tonicity modifier" as used herein denotes pharmaceutically acceptable tonicity agents that are used to modulate the tonicity of the formulation. The formulations of the present invention are preferably isosmotic, that is they have an osmotic pressure that is substantially the same as human blood serum. The tonicity modifiers used in the formulations are preferably non-ionic tonicity modifiers and are preferably selected from
30 the group consisting of mannitol, sucrose, glycerol, sorbitol and trehalose. A preferred non-ionic tonicity modifier is mannitol, e.g. D-mannitol. The concentration of the tonicity modifier will be dependent on the concentration of other components of the formulation, especially where the formulation is intended to be isosmotic. Typically, the non-ionic tonicity modifier will be employed at a concentration of about 90 mM to about 360 mM,
35 more preferably at a concentration of about 150 mM to about 250 mM, and most preferably at a concentration of about 230 mM. In preferred embodiments, the non-ionic tonicity modifier is mannitol at a concentration of about 90 mM to about 360 mM, more

preferably at a concentration of about 150 mM to about 250 mM, and most preferably at a concentration of about 230 mM.

5 Generally, the components and amounts of the liquid formulations are chosen to provide a formulation with a pH of about 6.6 to about 7.4, more preferably a pH of about 6.8 to about 7.2, and most preferably a pH of about 7.0. Arginine may be added quantum sufficit (q.s.) to adjust pH so that it is within a desired pH range. It is preferred that the pH adjustment is not done using hydrochloric acid or sodium hydroxide.

10 In one embodiment, the liquid formulations used in the present invention consist of the GLP-2 analogue at a concentration of about 2 mg/mL to about 30 mg/mL a buffer selected from the group consisting of a histidine buffer, mesylate buffer, acetate buffer, glycine buffer, lysine buffer, TRIS buffer, Bis-Tris buffer and MOPS buffer, the buffer being present at a concentration of about 5 mM to about 50 mM, a non-ionic tonicity
15 modifier selected from the group consisting of mannitol, sucrose, glycerol, sorbitol and trehalose at a concentration of about 90 mM to about 360 mM, arginine q.s. to provide a pH of about 6.6 to about 7.4.

In one embodiment, the liquid formulations used in the present invention consist of the
20 GLP-2 analogue at a concentration of about 2 mg/mL to about 30 mg/mL, a buffer selected from the group consisting of a histidine buffer, mesylate buffer and acetate buffer, the buffer being present at a concentration of about 5 mM to about 50 mM, a non-ionic tonicity modifier selected from the group consisting of mannitol, sucrose, glycerol and sorbitol at a concentration of about 90 mM to about 360 mM, arginine q.s. to provide
25 a pH of about 6.6 to about 7.4. Exemplary amounts of the GLP-2 analogue in the liquid formulations of the present invention are at about 5 mg/mL, at about 10 mg/mL or at about 20 mg/mL.

In a further embodiment, the liquid formulations used in the present invention comprise
30 the GLP-2 analogue at a concentration of about 20 mg/mL, histidine buffer at a concentration of about 15 mM, mannitol at a concentration of about 230 mM, and arginine q.s. to provide a pH of about 7.0.

In a further embodiment, the liquid formulations used in the present invention comprise
35 the GLP-2 analogue at a concentration of about 20 mg/mL, histidine buffer at a concentration of about 15 mM, mannitol at a concentration of about 230 mM and the pH is about 7.0.

In a further embodiment, the liquid formulations used in the present invention comprise the GLP-2 analogue at a concentration of about 5 mg/mL or 10 mg/mL, histidine buffer at a concentration of about 15 mM, mannitol at a concentration of about 230 mM, and
5 optionally 4 mM to 5 mM arginine q.s. where needed to adjust the pH to provide a pH of about 7.0.

In a further embodiment, the liquid formulations used in the present invention comprise ZP1848-acetate or H-HGEGTFSSSELATILDALAARDFIAWLIATKITDKKKKKK-NH₂
10 acetate (SEQ ID NO: 1) at a concentration of about 20 mg/mL, histidine buffer at a concentration of about 15 mM, mannitol at a concentration of about 230 mM and the pH is about 7.0.

In a further embodiment, the liquid formulations used in the present invention comprise
15 an acetate salt of a glucagon-like peptide 2 (GLP-2) analogue having the formula: (H-HGEGTFSSSELATILDALAARDFIAWLIATKITDKKKKKK-NH₂), x(CH₃COOH) where x is 1.0 to 8.0., at a concentration of about 20 mg/mL, histidine buffer at a concentration of about 15 mM, mannitol at a concentration of about 230 mM and the pH is about 7.0.

20 In a further embodiment, the liquid formulations used in the present invention comprise an acetate salt of a glucagon-like peptide 2 (GLP-2) analogue having the formula: (H-HGEGTFSSSELATILDALAARDFIAWLIATKITDKKKKKK-NH₂), x(CH₃COOH) where x is 1.0 to 8.0., at a concentration of about 20 mg/mL, histidine buffer at a concentration of about 15 mM, mannitol at a concentration of about 230 mM and the pH is about 7.0, in a
25 once or twice daily dosing regimen.

In a further embodiment, the liquid formulations used in the present invention comprise ZP1846 H-HGEGSFSSSELSTILDALAARDFIAWLIATKITDKKKKKK-NH₂ (SEQ ID NO: 7);
30 at a concentration of about 20 mg/mL, histidine buffer at a concentration of about 15 mM, mannitol at a concentration of about 230 mM, and arginine q.s. to provide a pH of about 7.0.

In a further embodiment, the liquid formulations used in the present invention comprise ZP1846 H-HGEGSFSSSELSTILDALAARDFIAWLIATKITDKKKKKK-NH₂ (SEQ ID NO: 7);
35 at a concentration of about 20 mg/mL, histidine buffer at a concentration of about 15 mM, mannitol at a concentration of about 230 mM and the pH is about 7.0.

In some cases, the liquid formulations further comprise a preservative. In some cases, the preservative is one selected from the group consisting of meta-cresol, phenol, benzalkonium chloride, chloro butanol, methyl paraben and potassium sorbate. Generally, the preservative is present in a concentration of about 0.1 % to about 1% of the final formulation volume or 1 mg/ml to 5 mg/ml of the formulation.

The liquid formulations according to the present invention are preferably an isosmotic liquid formulation. "Isosmotic" means that the formulations of the present invention have the same or a similar osmotic pressure with bodily fluids. Preferably, the formulations of the present invention have an osmolality of about 300 ± 60 mOsm as measured by an osmometer.

WO 2020/065064 further demonstrated that the formation of covalently linked oligomers of the GLP-2 analogue is inversely dependent on the concentration of the GLP-2 analogue in the formulation. As shown in the examples, this amount of covalently bonded oligomers can be determined using size exclusion chromatography and determining the area under the peaks for monomeric GLP-2 analogue and oligomers respectively. This can be done e.g. using a Dionex Ultimate3000 HPLC system, giving a linear gradient, at a flow rate of 0.5 mL/min was used for the analysis. The mobile phase consisted of 0.1% TFA in 45% acetonitrile and 55% Milli-Q water. A wavelength of 215 nm was used for detection. This means that the formulations of the present invention generally contain the GLP-2 analogue at a concentration of about 2 mg/mL to about 30 mg/mL, more preferably at a concentration of about 15 mg/mL to about 25 mg/mL, and most preferably at a concentration of about 20 mg/mL. In further embodiments, the present invention generally contain the GLP-2 analogue at a concentration of about 2 mg/mL, 5 mg/mL, 10 mg/mL or 20 mg/mL. In some aspects of the present invention, it is preferred that the concentration of the GLP-2 analogue is selected so that the formulation contains 10% or less, more preferably 5% or less, more preferably 4% or less, more preferably 3% or less, and more preferably 2% or less of the GLP-2 analogue in the form of covalently bonded oligomeric products, preferably after 18 months storage. By way of illustration, the amount of covalently bonded oligomeric product may be in the range of between 2% to 5%, more preferably in the range of between 2% to 4%, and most preferably in the range of between 2% to 3%. In a preferred embodiment, the range may be up to 3.5% (4%) after 30 months storage.

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In some cases, the formulation of the present invention may be used in a once or twice daily dosage regime. In some cases, the formulation of the present invention may be

used in a once or twice weekly dosage regime. Alternatively or additionally, the dosing regimen of the GLP-2 analogues of the present invention may comprise a plurality or course of doses separated in time by 2 days, 2.5 days, 3 days, 3.5 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days or 12 days. In a preferred embodiment, the doses are separated in time by 3 days, 3.5 days, 4 days, 5 days, 6 days, 7 days or 8 days. In a preferred embodiment, doses are separated in time by 3 days, 3.5 days, 4 days or 7 days. As will be appreciated in the art, the time between doses may be varied to some extent so that each and every dose is not separated by precisely the same time. This will often be directed under the discretion of the physician or specialist. Thus, doses may be separated in time by a clinically acceptable range of times, e.g. from about 2 days to about 10 days, or from about 3 or 4 days to about 7 or 8 days.

The formulations or the drug products of the present invention are stable liquid pharmaceutical formulations of GLP-2 analogues. A "stable" formulation is one in which the peptide therein essentially retains its physical stability and/or chemical stability and/or biological activity upon storage. Preferably, the formulation essentially retains its physical and chemical stability, as well as its biological activity upon storage. The storage period is generally selected based on the intended shelf-life of the formulation and is typically a consequence of the degradation speed of the components of the formulation. The formulations of the present invention are provided as stable liquid formulations, e.g. stable aqueous liquid formulations. Various analytical techniques for measuring protein stability are available in the art and are reviewed in *Peptide and Protein Drug Delivery*, 247-301, Vincent Lee Ed., Marcel Dekker, Inc., New York, N.Y., Pubs. (1991) and Jones, A. *Adv. Drug Delivery Rev.* 10: 29-90 (1993), for example.

In the present invention, "stable" formulations include formulations in which at least 80%, more preferably at least 90%, more preferably at least 95%, more preferably at least 96%, more preferably at least 97%, more preferably at least 98%, and most preferably at least 99% of the content of the drug product (% label claim) is active in the formulation after it has been stored under refrigerated conditions, typically at 2-8°C. Stable formulations lead to longer shelf life of the drug product.

Stability can be measured as degradation at a selected temperature for a selected time period, for example using elevated temperature to reduce the period over which a formulation is tested. Generally, storage at a temperature between 2 to 8°C denotes storage under normal refrigerated conditions. In certain embodiments, the formulation is stable and a given shelf life is achieved under such conditions for at least 6 months,

preferably at least 12 months, more preferably at least 18 months, more preferably at least 24 months, more preferably at least 30 months, or more preferably at least 36 months.

- 5 Stability can be evaluated qualitatively and/or quantitatively in a variety of different ways, including evaluation of aggregate formation (e.g. using size exclusion chromatography, by measuring turbidity, and/or by visual inspection); by evaluating impurities by UHPLC; amino-terminal or carboxy-terminal sequence analysis; mass spectrometric analysis; evaluating biological activity by potency assays; etc. Degradation may involve any one or more of: Aggregation, deamidation (e.g. Asn deamidation), oxidation (e.g. Met oxidation), isomerization (e.g. Asp isomerisation), clipping/hydrolysis/fragmentation, succinimide formation, unpaired cysteine(s), N-terminal extension, C-terminal processing, etc. Until the results described in the examples below, it was believed that isomerization leading to isoAsp(33) degradation products led to loss of biological activity.

- 15 The potency data in the examples shows that the degradation product isoAsp(33) ZP1848 is equally or more potent than the main compound ZP1848 and the metabolite of isoAsp(33) is equally potent to isoAsp(33). IsoAsp(33) ZP1848 is therefore considered as an active related substance and is contributing to the activity of the pharmacodynamic effects of ZP1848/glepaglutide drug product on the GLP-2 receptor (see Table 2). As demonstrated in the examples, the content of active drug substance in the drug product used in the present invention refers to the amount of GLP-2 analogue, such as ZP1848 and isoAsp(33) ZP1848.

- 25 In the present context, the term “content” is used for the amount (mg/mL) of ZP1848/glepaglutide and isoAsp(33) ZP1848 in the drug product. A drug product has a nominal/declared content which could be 20 mg/mL and is named “label claim”. Thus, the content of a given drug product batch can be expressed as % label claim. Content may be measured in a chemical analysis and expressed as percentage of the labelled drug product (% label claim). Thus, content calculation (% label claim) can be expressed using the following formula:

$$\text{Content (\% label claim)} = \frac{\text{Content ZP1848} \left(\frac{\text{mg}}{\text{mL}} \right) + \text{Content isoAsp(33)} \left(\frac{\text{mg}}{\text{mL}} \right)}{\text{Label claim} \frac{\text{mg}}{\text{mL}}} \times 100$$

- 35 To accommodate the degradation observed during storage, a lower shelf-life acceptance criterion of assay of not less than 90% of label claim is generally proposed. A shelf-life

acceptance criterion is a limit for a tested parameter that a batch should comply to during storage of the defined shelf-life of the product.

If isoAsp(33) is included in the content of the drug product, its contribution to the total
5 content increases over time, as the content of isoAsp(33) increases while the content of
ZP1848 decreases. This has the benefit of extending the shelf-life with additional
months as shown in Table 5 and Figure 1 in the examples, in which the additional
months, which can be added to the self-life of an aqueous formulation comprising
ZP1848 only, are theoretically calculated. Thus, in preferred embodiments, the shelf life
10 can be extended with 5 months, such as preferably 11 months, such as preferably 16
months, such as preferably 22 months, more preferably 27 months. These numbers are
based on an example of % allowed degradation during shelf life of ZP1848 and
isoAsp(33), e.g., 1%, 2%, 3%, 4%, and 5% respectively, and calculation of content, when
the drug product is stored at 2-8°C. This is possible because the drop in content is
15 slower and it takes thus longer time for the drug product to reach the specification limits
for the content of peptide, i.e. e.g. 90% lower acceptance limit of content at end of shelf-
life.

In addition, based on the results in Table 3 and 4, it is possible to calculate the
20 concentration ranges for formulations with different peptide concentrations of ZP1848 (2
mg/mL, 5 mg/mL, 10 mg/mL, 20 mg/mL) at the beginning, middle and end of shelf life
based on an end of shelf life of the formulation at 90% potency with no more than 10% of
that potency was accounted for by isoAsp33 ZP1848. These ranges are set out in Table
7 and show decreasing ZP1848 concentration over time and increasing concentration of
25 isoAsp(33) ZP1848. This end of shelf life calculation defines a lowest concentration
value for ZP1848 and a highest concentration value for isoAsp(33) ZP1848. As shown in
Table 3, isoAsp(33) constitutes approximately 50% of the total of the degradation
products, contributing to the drug content of the formulations, determined as % of label
claim with estimated 1.4% of the content after 12 months, 2.1% of the content after 18
30 months 2.6% of the content after 24 months, 3.3% of the content after 30 months, 3.5%
of the content after 36 months at 2-8°C (absolute values). This in turn means that the
shelf life of aqueous formulations of ZP1848 may be extended significantly. Accordingly,
isoAsp(33) ZP1848 may constitute from about 0.1% to about 10.0% of total drug
substance in a formulation based on the shelf life of the formulation being exceeded
35 when the drug content of the formulation is reduced to about 90% of the initial content.

For an initial peptide concentration of 2 mg/mL, the present invention provides GLP-2 analogues for use in therapy or a method of treating a stomach and bowel related disorder in a subject in need thereof, the therapy or method comprising administering to the subject stable liquid pharmaceutical formulation comprising 1.9 ± 0.1 mg/mL of a mixture of active glucagon-like peptide 2 (GLP-2) analogues selected from:

- (i) a first GLP-2 analogue having the formula H-HGEGTFSSELATILDALAARDFIAWLIATKIT-[Iso-Asp]-KKKKKK-NH₂ (SEQ ID NO: 4), or a pharmaceutically acceptable salt thereof, in an amount of from 0.01 to 0.18 mg/mL; and
- (ii) a second GLP-2 analogue having the formula H-HGEGTFSSELATILDALAARDFIAWLIATKITDKKKKK-NH₂ (SEQ ID NO: 1), or a pharmaceutically acceptable salt thereof, in an amount of from 1.62 to 1.99 mg/mL.

In this embodiment, at the beginning of the shelf life of the product, the formulation comprises the first GLP-2 analogue in an amount of 0.01 to 0.05 mg/mL and the second GLP-2 analogue in an amount of 1.85 to 1.99 mg/mL.

In this embodiment, at the middle of the shelf life of the product, the formulation comprises the first GLP-2 analogue in an amount of 0.05 to 0.15 mg/mL and the second GLP-2 analogue in an amount of 1.75 to 1.90 mg/mL.

In this embodiment, at the end of the shelf life of the product, the formulation comprises the first GLP-2 analogue in an amount of 0.10 to 0.18 mg/mL and the second GLP-2 analogue in an amount of 1.62 to 1.85 mg/mL.

For an initial peptide concentration of 5 mg/mL, the present invention provides GLP-2 analogues for use in therapy or a method of treating a stomach and bowel related disorder in a subject in need thereof, the therapy or method comprising administering to the subject stable liquid pharmaceutical formulation comprising 4.7 ± 0.5 mg/mL of a mixture of active glucagon-like peptide 2 (GLP-2) analogues selected from:

- (i) a first GLP-2 analogue having the formula H-HGEGTFSSELATILDALAARDFIAWLIATKIT-[Iso-Asp]-KKKKKK-NH₂ (SEQ ID NO: 4), or a pharmaceutically acceptable salt thereof, in an amount of from 0.01 to 0.45 mg/mL; and
- (ii) a second GLP-2 analogue having the formula H-HGEGTFSSELATILDALAARDFIAWLIATKITDKKKKK-NH₂ (SEQ ID NO: 1), or a pharmaceutically acceptable salt thereof, in an amount of from 4.05 to 4.99 mg/mL.

In this embodiment, at the beginning of the shelf life of the product, the formulation comprises the first GLP-2 analogue in an amount of 0.01 to 0.15 mg/mL and the second GLP-2 analogue in an amount of 4.60 to 4.99 mg/mL.

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In this embodiment, at the middle of the shelf life of the product, the formulation comprises the first GLP-2 analogue in an amount of 0.10 to 0.35 mg/mL and the second GLP-2 analogue in an amount of 4.25 to 4.70 mg/mL.

10 In this embodiment, at the end of the shelf life of the product, the formulation comprises the first GLP-2 analogue in an amount of 0.25 to 0.45 mg/mL and the second GLP-2 analogue in an amount of 4.05 to 4.30 mg/mL.

For an initial peptide concentration of 10 mg/mL, the present invention provides GLP-2 analogues for use in therapy or a method of treating a stomach and bowel related disorder in a subject in need thereof, the therapy or method comprising administering to the subject stable liquid pharmaceutical formulation comprising 9.30 ± 1 mg/mL of a mixture of active glucagon-like peptide 2 (GLP-2) analogues selected from:

- 15 (i) a first GLP-2 analogue having the formula H-HGEGTFSSELATILDALAARDFIAWLIATKIT-[Iso-Asp]-KKKKKK-NH₂ (SEQ ID NO: 4), or a pharmaceutically acceptable salt thereof, in an amount of from 0.01 to 0.9 mg/mL; and
- 20 (ii) a second GLP-2 analogue having the formula H-HGEGTFSSELATILDALAARDFIAWLIATKITDKKKKK-NH₂ (SEQ ID NO: 1), or a pharmaceutically acceptable salt thereof, in an amount of from 8.10 to 9.99 mg/mL.

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In this embodiment, at the beginning of the shelf life of the product, the formulation comprises the first GLP-2 analogue in an amount of 0.01 to 0.55 mg/mL and the second GLP-2 analogue in an amount of 9.20 to 9.99 mg/mL.

30 In this embodiment, at the middle of the shelf life of the product, the formulation comprises the first GLP-2 analogue in an amount of 0.35 to 0.75 mg/mL and the second GLP-2 analogue in an amount of 8.60 to 9.30 mg/mL.

In this embodiment, at the end of the shelf life of the product, the formulation comprises the first GLP-2 analogue in an amount of 0.50 to 0.90 mg/mL and the second GLP-2 analogue in an amount of 8.10 to 8.70 mg/mL.

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For an initial peptide concentration of 20 mg/mL, the present invention provides GLP-2 analogues for use in therapy or a method of treating a stomach and bowel related disorder in a subject in need thereof, the therapy or method comprising administering to the subject stable liquid pharmaceutical formulation comprising 18.5 ± 2 mg/mL of a mixture of active glucagon-like peptide 2 (GLP-2) analogues selected from:

(i) a first GLP-2 analogue having the formula H-HGEGTFSSELATILDALAARDFIAWLIATKIT-[Iso-Asp]-KKKKKK-NH₂ (SEQ ID NO: 4), or a pharmaceutically acceptable salt thereof, in an amount of from 0.01 to 1.8 mg/mL; and

(ii) a second GLP-2 analogue having the formula H-HGEGTFSSELATILDALAARDFIAWLIATKITDKKKKK-NH₂ (SEQ ID NO: 1), or a pharmaceutically acceptable salt thereof, in an amount of from 16.20 to 19.99 mg/mL.

In this embodiment, at the beginning of the shelf life of the product, the formulation comprises the first GLP-2 analogue in an amount of 0.01 to 0.90 mg/mL and the second GLP-2 analogue in an amount of 18.20 to 19.99 mg/mL.

In this embodiment, at the middle of the shelf life of the product, the formulation comprises the first GLP-2 analogue in an amount of 0.75 to 1.10 mg/mL and the second GLP-2 analogue in an amount of 17.30 to 18.50 mg/mL.

In this embodiment, at the end of the shelf life of the product, the formulation comprises the first GLP-2 analogue in an amount of 0.90 to 1.80 mg/mL and the second GLP-2 analogue in an amount of 16.20 to 17.50 mg/mL.

In further embodiments, the shelf life of aqueous formulations of ZP1848 may be extended significantly to the shelf-life of an aqueous formulations only comprising ZP1848 with at least from 5 to 10 months at 2-8°C, such as at least from 10 to 15 months at 2-8°C, such as at least from 15 to 20 months at 2-8°C, such as at least from 20 to 25 months at 2-8°C, such as at least from 25 to 30 months at 2-8°C storage conditions, when the both the content of ZP1848 and the content of isoAsp(33) is taken into account.

In certain embodiments, when taken both the content of ZP1848 and the content of isoAsp(33) into account, the formulation is stable under storage at a temperature between 2 to 8°C for at least 11 months, preferably for at least 22 months, more preferably at least 32 months, more preferably at least 43 months, more preferably at least 54 months, more preferably at least 65 months, and more preferably at least 76 months.

In accordance with the present invention, due to the activity of the isoAsp(33) degradation product, the drug product may first be stored at 2 to 8°C for a certain number of months and then for a certain number of days or weeks at room temperature at around 20°C, 25°C or 30°C. This storage under room temperature (or consecutive storage or dual storage or in-use period or alternative storage condition) has the advantage that patients are granted more flexible storage and handling conditions when using the drug or drug product, because they can leave the drug product outside the refrigerator for a certain number of days or weeks (e.g. when travelling) without affecting the safety, efficacy and/or quality of the drug product.

In preferred embodiments, the liquid aqueous formulation, after having been stored for a certain number of months at 2 to 8°C, may then be kept for preferably 1 week, more preferably for 2 weeks at room temperatures at around 20°C, 25°C or 30°C.

In preferred embodiments, the liquid aqueous formulation, after having been stored for a certain number of months at 2 to 8°C, may then be kept for a certain number of days or weeks at room temperatures between 20°C to 30°C, preferably at room temperatures between 20°C to 25°C, more preferably at room temperatures between 25°C to 30°C.

In preferred embodiments, the liquid aqueous formulation, after having been stored for a certain number of months at 2 to 8°C, may then be kept for preferably 14 days, more preferably for 1 week, more preferably for 2 weeks at room temperatures between 20°C to 30°C, preferably at room temperatures between 20°C to 25°C, more preferably at room temperatures between 25°C to 30°C.

In further embodiments, the formulation may be stored at 2 to 8°C for at least 6 months, more preferably for at least 12 months, more preferably at least 18 months, more preferably at least 24 months, more preferably at least 30 months, more preferably at least 36 months, and then kept then be kept for preferably 14 days, more preferably for 1 week, more preferably for 2 weeks at room temperatures at around 20°C, 25°C or 30°C.

In further embodiments, the formulation may be stored at 2 to 8°C for at least 6 months, more preferably for at least 12 months, more preferably at least 18 months, more preferably at least 24 months, more preferably at least 30 months, more preferably at least 36 months, and then be kept for a certain number of days or weeks at room temperatures between 20°C to 30°C, preferably at room temperatures between 20°C to

25°C, more preferably at room temperatures between 25°C to 30°C.

In further embodiments, the formulation may be stored at 2 to 8°C for at least 6 months, more preferably for at least 12 months, more preferably at least 18 months, more
5 preferably at least 24 months, more preferably at least 30 months, more preferably at least 36 months, then be kept for preferably 14 days, more preferably for 1 week, more preferably for 2 weeks at room temperatures between 20°C to 30°C, preferably at room temperatures between 20°C to 25°C, more preferably at room temperatures between
10 25°C to 30°C.

In further embodiments, when taken both the content of ZP1848 and the content of isoAsp(33) into account, the formulation may be stored at a temperature between 2 to 8°C for at least 11 months, preferably for at least 22 months, more preferably at least 32 months, more preferably at least 43 months, more preferably at least 54, and then be
15 kept for preferably 1 week, more preferably for 2 weeks at room temperatures at around 20°C, 25°C or 30°C.

In further embodiments, when taken both the content of ZP1848 and the content of isoAsp(33) into account, the formulation may be stored at a temperature between 2 to 8°C for at least 11 months, preferably for at least 22 months, more preferably at least 32 months, more preferably at least 43 months, more preferably at least 54, then may be
20 kept for a certain number of days or weeks at room temperatures between 20°C to 30°C, preferably at room temperatures between 20°C to 25°C, more preferably at room temperatures between 25°C to 30°C.

In further embodiments, when taken both the content of ZP1848 and the content of isoAsp(33) into account, the formulation may be stored at a temperature between 2 to 8°C for at least 11 months, preferably for at least 22 months, more preferably at least 32 months, more preferably at least 43 months, more preferably at least 54, and then may
30 be kept for preferably for 1 week, more preferably for 2 weeks at room temperatures between 20°C to 30°C, preferably at room temperatures between 20°C to 25°C, more preferably at room temperatures between 25°C to 30°C.

A peptide "retains its physical stability" in a pharmaceutical formulation if it shows no sign
35 or insignificant signs of aggregation, precipitation and/or denaturation upon e.g. visual examination of colour and/or clarity, or as e.g. measured by UV light scattering, dynamic light scattering, circular dichroism, light obscuration, flow imaging techniques, or by size

exclusion chromatography and is considered to still retain its biological activity.

5 A peptide "retains its chemical stability" in a pharmaceutical formulation, if the chemical stability at a given time is such that the peptide is considered to still retain its biological activity as defined below. Chemical stability can be assessed by detecting and
quantifying chemically altered forms of the peptide. Chemical alteration may involve isomerization, oxidation, size modification (e.g. clipping) which can be evaluated using UHPLC or size exclusion chromatography, mass spectrometry, for example. Other types
10 of chemical alteration include charge alteration (e.g. occurring as a result of deamidation) which can be evaluated by UHPLC or ion-exchange chromatography.

In a further embodiment, the liquid formulation is selected from the group consisting of an aqueous liquid formulation, a liquid formulation in various hydrophilic or hydrophobic solvents, an emulsion and a liquid suspension. In a preferred embodiment, the liquid
15 formulation is an aqueous liquid formulation.

By way of example, the liquid formulations of the present invention may be prepared by mixing stock solutions of the GLP-2 analogue, the buffer, the non-ionic tonicity modifier and optionally the preservative in water, optionally diluting the resulting solution and
20 adjusting to the target pH. Conveniently, the solutions of the buffer and the non-ionic tonicity modifier may first be mixed to provide a desired concentration of each excipient. The solution of the GLP-2 analogue may then be added, and if necessary the pH adjusted, for examples using acetic acid/0.5 M L-arginine. Water was added up to the final volume. This may be referred to as a two-pot process for making the GLP-2
25 analogue formulation. In an alternative one-pot process described in WO 2023/036862, the excipients present in the formulation were first mixed with a first volume of water for injection to provide an excipient solution at 70-90% of the volume of a final liquid formulation, then the GLP-2 analogue drug substance was added to the excipient solution to provide a solution of the excipients and the GLP-2 analogue at 70-90% of the
30 volume of a final liquid formulation, and finally the pH and the volume of the liquid composition were adjusted as needed to provide a liquid formulation at 100% of the volume of the final liquid pharmaceutical formulation.

Preferably, the glucagon-like peptide 2 (GLP-2) analogues are administered to patients
35 parenterally, preferably by injection, most typically by subcutaneous injection, intramuscular injection, intravenous injection or intraperitoneal injection. Administration by subcutaneous injection is preferred. The injection may be carried out by a physician,

specialist, nurse or other healthcare professional, or may be self-administered by the patient. As set out herein, in some aspects, the formulations of the present invention have a viscosity that facilitates loading of the formulation into a pre-filled syringe, an injection pen, auto-injector or other injector device. This may have the advantage of pre-determining the dose of the formulation administered to the patient, e.g. without the need for measurement from a multi-use vial. Accordingly, in other aspects, the present invention provides an article of manufacture or a kit comprising a container holding the stable, such as e.g. an aqueous stable pharmaceutical formulation of the GLP-2 analogue used in the present invention or a pre-filled syringe or injector device or injector pen containing an aqueous liquid formulation comprising the GLP-2 analogue used in the present invention.

Medical Conditions

The GLP-2 analogue formulations of the present invention are useful as a pharmaceutical agent for preventing or treating an individual suffering from gastrointestinal disorders, including the upper gastrointestinal tract of the oesophagus by administering an effective amount of a GLP-2 analogue, or a salt thereof as described herein. The stomach and intestinal-related disorders include ulcers of any aetiology (e.g., peptic ulcers, drug-induced ulcers, ulcers related to infections or other pathogens), digestion disorders, malabsorption syndromes, short-bowel syndrome, cul-de-sac syndrome, inflammatory bowel disease, celiac sprue (for example arising from gluten induced enteropathy or celiac disease), tropical sprue, hypogammaglobulinemic sprue, enteritis, ulcerative colitis, small intestine damage, and chemotherapy induced diarrhoea/mucositis (CID). In the present invention, the uses of the GLP-2 analogue formulation includes the prevention or treatment of stomach and bowel-related disorders such as ulcers, digestion disorders, malabsorption syndromes, short-gut syndrome, cul-de-sac syndrome, inflammatory bowel disease, celiac sprue (for example arising from gluten induced enteropathy or celiac disease), tropical sprue, hypogammaglobulinemic sprue, enteritis, regional enteritis (Crohn's disease), ulcerative colitis, small intestine damage or short bowel syndrome (SBS). In preferred uses, the stomach and bowel-related disorder is short bowel syndrome.

As mentioned above, in general individuals who would benefit from increased small intestinal mass and consequent and/or maintenance of normal small intestine mucosal structure and function are candidates for treatment with the present GLP-2 analogues. Particular conditions that may be treated with GLP-2 analogue include the various forms of sprue including celiac sprue which results from a toxic reaction to alpha-gliadin from

heat and may be a result of gluten-induced enteropathy or celiac disease, and is marked by a significant loss of villi of the small bowel; tropical sprue which results from infection and is marked by partial flattening of the villi; hypogammaglobulinemic sprue which is observed commonly in patients with common variable immunodeficiency or

5 hypogammaglobulinemia and is marked by significant decrease in villus height. The therapeutic efficacy of the GLP-2 analogue treatment may be monitored by enteric biopsy to examine the villus morphology, by biochemical assessment of nutrient absorption, by patient weight gain, or by amelioration of the symptoms associated with these conditions.

10

Another particular condition which may be treated with the GLP-2 analogues of the invention, or for which the GLP-2 analogues may be useful therapeutically and/or prophylactically is short bowel syndrome (SBS), also known as short gut syndrome or simply short gut, which results from surgical resection, congenital defect or disease-associated loss of absorption in the bowel in which patients are subsequently unable to maintain fluid, electrolyte, and nutrient balances on a conventional diet. Despite an adaptation that occurs generally in the two years after resection, SBS patients have reduced dietary uptake and fluid loss.

15

Other conditions that may be treated with the GLP-2 analogues of the invention, or for which the GLP-2 analogues may be useful prophylactically, include in addition to the above mentioned radiation enteritis, infectious or post-infectious enteritis, and small intestinal damage due to cancer-chemotherapeutic or toxic agents.

20

The GLP-2 analogues may also be used for the treatment of malnutrition, for example cachexia and anorexia.

25

A particular embodiment of the invention is concerned with using the present peptides for the prevention and/or treatment of intestinal damage and dysfunction. Such damage and dysfunction is a well-known side effect of cancer-chemotherapy treatment.

30

Chemotherapy administration is frequently associated with unwanted side effects related to the gastrointestinal system such as mucositis, diarrhoea, bacterial translocation, malabsorption, abdominal cramping, gastrointestinal bleeding and vomiting. These side effects are clinical consequences of the structural and functional damage of the intestinal epithelium and frequently make it necessary to decrease the dose and frequency of chemotherapy.

35

- Administration of the present GLP-2 peptide analogues may enhance trophic effect in the intestinal crypts and rapidly provide new cells to replace the damaged intestinal epithelium following chemotherapy. The ultimate goal achieved by administering the present peptides is to reduce the morbidity related to gastrointestinal damage of patients
- 5 undergoing chemotherapy treatment while creating the most optimal chemotherapy regime for the treatment of cancer. Concomitant prophylactic or therapeutic treatment may be provided in accordance with the present invention to patients undergoing or about to undergo radiation therapy.
- 10 The stem cells of the small intestinal mucosa are particularly susceptible to the cytotoxic effects of chemotherapy due to their rapid rate of proliferation (Keefe et al., Gut, 47: 632-7, 2000). Chemotherapy-induced damage to the small intestinal mucosa is clinically often referred to as gastrointestinal mucositis and is characterized by absorptive and barrier impairments of the small intestine. For example, it has been shown that, the
- 15 broadly used chemotherapeutic agents, 5-FU, irinotecan and methotrexate increase apoptosis leading to villus atrophy and crypt hypoplasia in the small intestine of rodents (Keefe et al., Gut 47: 632-7, 2000; Gibson et al., J Gastroenterol. Hepatol. Sep;18(9):1095-1100, 2003; Tamaki et al., J. Int. Med. Res. 31(1):6-16, 2003). Chemotherapeutic agents have been shown to increase apoptosis in intestinal crypts at
- 20 24 hours after administration and subsequently to decrease villus area, crypt length, mitotic count per crypt, and enterocyte height three days after chemotherapy in humans (Keefe et al., Gut, 47: 632-7, 2000). Thus, structural changes within the small intestine directly lead to intestinal dysfunction and in some cases diarrhoea.
- 25 Gastrointestinal mucositis after cancer chemotherapy is an increasing problem that is essentially untreatable once established, although it gradually remits. Studies conducted with the commonly used cytostatic cancer drugs 5-FU and irinotecan have demonstrated that effective chemotherapy with these drugs predominantly affects structural integrity and function of the small intestine while the colon is less sensitive and mainly responds
- 30 with increased mucus formation (Gibson et al., J. Gastroenterol. Hepatol. Sep;18(9):1095-1100, 2003; Tamaki et al., J Int. Med. Res. 31(1):6-16, 2003).
- The formulations of the present invention comprising GLP-2 analogues may be useful in the prevention and/or treatment of gastrointestinal injury and side effects of
- 35 chemotherapeutic agents. This potentially important therapeutic application may apply to currently used chemotherapeutic agents such as but not limited to: 5-FU, Altretamine, Bleomycin, Busulfan, Capecitabine, Carboplatin, Carmustine, Chlorambucil, Cisplatin,

- Cladribine, Crisantaspase, Cyclophosphamide, Cytarabine, Dacarbazine, Dactinomycin, Daunorubicin, Docetaxel, Doxorubicin, Epirubicin, Etoposide, Fludarabine, Fluorouracil, Gemcitabine, Hydroxycarbamide, Idarubicin, Ifosfamide, Irinotecan, Liposomal doxorubicin, Leucovorin, Lomustine, Melphalan, Mercaptopurine, Mesna, Methotrexate,
- 5 Mitomycin, Mitoxantrone, Oxaliplatin, Paclitaxel, Pemetrexed, Pentostatin, Procarbazine, Raltitrexed, Streptozocin, Tegafur-uracil, Temozolomide, Thiotepa, Tioguanine/Thioguanine, Topotecan, Treosulfan, Vinblastine, Vincristine, Vindesine, Vinorelbine, Bleomycin, Busulfan, Capecitabine, Carboplatin, Carmustine, Chlorambucil, Cisplatin, Cladribine, Crisantaspase, Cyclophosphamide, Cytarabine, Dacarbazine,
- 10 Dactinomycin, Daunorubicin, Docetaxel, Doxorubicin, Epirubicin, Etoposide, Fludarabine, Fluorouracil, Gemcitabine, Hydroxycarbamide, Idarubicin, Ifosfamide, Irinotecan, Liposomal doxorubicin, Leucovorin, Lomustine, Melphalan, Mercaptopurine, Methotrexate, Mitomycin, Mitoxantrone, Oxaliplatin, Paclitaxel, Pemetrexed, Pentostatin, Procarbazine, Raltitrexed, Streptozocin, Tegafur-uracil, Temozolomide, Thiotepa,
- 15 Tioguanine/Thioguanine, Topotecan, Treosulfan, Vinblastine, Vincristine, Vindesine, and Vinorelbine.

Delivery of the Formulations

- In some aspects, the present invention relates to a ready-to-use formulation of GLP-2 analogues, intended for parenteral administration, and suitable for use in e.g. vials, pre-filled syringes, infusion pumps, wearable injectors, disposable auto-injectors or adjustable dose auto-injectors. Typically, the glucagon-like peptide 2 (GLP-2) analogue are administered to patients parenterally, preferably by injection, most typically by subcutaneous injection, intramuscular injection, intravenous injection or intraperitoneal
- 20 injection. Administration by subcutaneous injection is preferred. The injection may be carried out by a physician, nurse or other healthcare professional, or may be self-administered by the patient. As set out herein, in some aspects, the formulations of the present invention have a viscosity that facilitates loading of the formulation into a pre-filled syringe, an injection pen or other injector device. This may have the advantage of pre-determining the dose of the formulation administered to the patient, e.g. without the
- 30 need for measurement from a multi-use vial. Accordingly, in other aspects, the present invention provides an article of manufacture or a kit comprising a container holding the stable, such as e.g. an aqueous stable pharmaceutical formulation of the GLP-2 analogue according to the present invention or a pre-filled syringe or injector device or
- 35 injector pen containing an aqueous liquid formulation comprising the GLP-2 analogue according to the present invention.

Examples

The following examples are provided to illustrate preferred aspects of the invention and are not intended to limit the scope of the invention. The GLP-2 analogues administered according to the dosage regimes described herein can be made according to the

5 methods such as solid phase peptide synthesis described in WO 2006/117565 or as described in WO 2020/065064, the contents of which are expressly incorporated by reference in its entirety.

Example 1: Chemical assessment of the formation of isoAsp(33) degradation product of ZP1848

This example describes the results from a forced degradation study conducted on the ZP1848 drug product, followed by analysis using a UHPLC analytical procedure for the determination of degradation products of ZP1848 drug product.

15 The purpose of this example was also to evaluate under which conditions the major degradation products and especially isoAsp(33) are formed.

The conditions tested were elevated temperature (25°C), elevated temperature and different pH conditions (pH 4, pH 7 and pH 8) . The intended storage condition of

20 ZP1848 drug product is between 2-8°C and with no exposure to light.

Materials and Methods

Formulations of ZP1848 were prepared at concentrations of 20 mg/mL and 2 mg/mL peptide as follows. A 50 mg/mL stock solution of ZP1848 was prepared. The formulation

25 consisted of 15 mM histidine and 260 mM mannitol and the formulation pH was set using 5 mM arginine and/or 0.4 mM acetic acid. All conditions were tested after 4 weeks.

Example 6 below shows that the peak of isoAsp(33) is eluting in the chromatogram with a retention different from ZP1848, a study was conducted with an amount of synthesized

30 isoAsp(33) that was added to a ZP1848 drug product sample and tested in the UHPLC method. The retention time of isoAsp(33) relative to ZP1848 was determined to 1.15 (see Table 6). This meant that although isoAsp(33) has same mass as ZP1848, the degradation product could be identified and detected in the presence of ZP1848.

UHPLC method

The samples were analyzed by reverse phase-UHPLC (RP-UHPLC) on a C18 column

with gradient elution using a trifluoroacetic acid/water/acetonitrile eluent system. The area-percentage (area-%) of the main peak and degradation products were determined by UV spectroscopy at 220 nm at each sampling time point. Purity is given as 100% minus the total sum of impurities (% weight/%weight).

5

Temperature stress

A sample was stored at room temperature. Additionally, a sample was stored in the dark at 2-8°C as a control.

10 pH stress

Additionally to formulations being formulated at pH 7, the 20 mg/mL drug product was also formulated at pH 4 and pH 8 and then stored at room temperature.

Results

15 A freshly prepared sample was compared to a sample (ZP1848 drug product) stored at 2-8°C for 4 weeks. Each stressed condition was also compared to the freshly prepared samples. The results are summarized in Table 1.

Table 1: Formation of the isoAsp(33) degradation product and purity for 2 mg/ml and 20 mg/ml drug product of ZP1848.

20

Sample	pH	20 mg/mL		2 mg/mL	
		% area isoAsp(33) (RRT ~1.15) formation	Purity%	% area isoAsp(33) (RRT ~1.15) formation	Purity%
Freshly prepared sample, start	7	0.37	97.48	0.41	97.37
4 weeks, 2-8°C, dark (control)	7	0.47	97.47	0.41	97.51
4 weeks, room temperature	7	1.17	96.64	1.10	94.94
4 weeks, room temperature	4	2.14	92.22	Not tested	
4 weeks, room temperature	8	1.62	94.09	Not tested	

Evaluation of 2 mg/mL drug product*Control*

In the chromatogram of an unstressed sample of 2 mg/mL (purity of freshly prepared ZP1848 drug product was 97.37%) and that no significant increase in degradation products were seen at 4 weeks at 2-8°C as purity was 97.51% (Table 1).

Temperature Stress

After 4 weeks during dark conditions at room temperature (approximately 21°C) a change was seen in some of the degradation peaks and purity is 94.94%. IsoAsp(33) increased to 1.10% (Table 1).

Summary for 2 mg/mL drug product

The study of 2 mg/mL ZP1848 drug product showed that the product is sensitive to higher temperature, as purity decreases significantly during 4 weeks. The amount of isoAsp(33) increases at all stressed conditions, but show no increase at 2-8°C.

Evaluation of 20 mg/mL drug product*Control*

In the freshly prepared sample of 20 mg/mL, the initial purity of the ZP1848 drug product was 97.48,% and that no significant increase in degradation products were seen at 4 weeks at 2-8°C as purity was 97.47% (Table 1).

Temperature stress (dark)

After 4 weeks during dark conditions at room temperature (approximately 21°C) a change was seen in some of the peaks and purity is 96.64%. The main differences are seen in peaks already present at start of the study but with increase in percentage after 4 weeks. The major peaks that changes is the isoAsp(33) peak. The amount of isoAsp(33) is 1.17% (Table 1). It is known from previous studies that 20 mg/mL is more stable than 2 mg/mL, and this could explain why the 2 mg/mL formulation was degraded to a higher extent for the same conditions.

pH stress

At room temperature, pH 4, a significant change was seen in the degradation products and purity is at 92.22% (Table 1). The increase in isoAsp(33) is significant and the amount of isoAsp(33) was 2.14%.

At room temperature with formulation at pH 8, a significant change can be seen in the detected degradation products and a purity of 94.09% (Table 1). The major increase are seen in the isoAsp(33) peak. The level of isoAsp(33) is 1.62%.

5 *Summary for 20 mg/mL drug product*

The study of 20 mg/mL ZP1848 drug product showed that products are sensitive to higher temperature and pH (pH 4 and 8, respectively) as purity decreases significantly during 4 weeks. The amount of isoAsp(33) increases at all stressed conditions but show little increase at 2-8°C during the 4 weeks study.

10

Conclusions for evaluation of the 2 mg/mL and 20 mg/mL drug product

The degradation of ZP1848 to isoAsp(33) was pronounced under all tested conditions, except when stored at 2-8°C during this 4 weeks study where degradation is small or is not observed. The formation of isoAsp(33) in liquid aqueous formulations became more significant over time at all storage conditions. Formation of isoAsp(33) from the isomerization of aspartate is a well-known mechanism for peptide degradation, but isoaspartate degradation products are usually not active.

15

Example 2: Determination of the potency of the major degradation product

20 **isoAsp(33)**

The purpose of this example was to investigate whether the degradation product isoAsp(33) and its major metabolite had any activity on the GLP-2 receptor, and if so, how potent it was. The ZP1848, its metabolites and the degradation products observed in Example 1 were newly synthesized for testing in this example.

25

Materials and Methods

GLP-2 R EC50 measurements

Generation of a human GLP-2 R expressing cell line

The cDNA encoding the human GLP-2 R was cloned from the image clone 5363415 (11924-117). The DNA encoding the GLP-2 R was amplified by PCR using primers encoding terminal restriction sites for subcloning. The 5'-end primer additionally encoded a Kozak consensus sequence to ensure efficient translation. The fidelity of the DNA encoding the GLP-2 R was confirmed by DNA sequencing. The PCR product encoding the GLP-2 R was subcloned into a mammalian expression vector containing a neomycin (G418) resistance marker.

30

35

The mammalian expression vector encoding the GLP-2 R was transfected into HEK293 cells by Lipofectamine PLUS transfection method and 24 hours after transfection cells were seeded for limited dilution cloning and selected with 500 µg/mL G418 in the culture medium. Four weeks later pooled colonies of GLP-2 R expressing cells were picked for
5 FACS sorting resulting in selection of a GLP-2 R expressing pool clone to be used for compound profiling in a GLP-2 R efficacy assay.

GLP-2 R efficacy assay

GLP-2R activity was measured in HEK293 cells stably expressing the human GLP-2 R
10 by quantitation of the accumulation of cAMP using the cAMP dynamic 2 assay kit from Cisbio (Cisbio #62AM4PEC) based on the HTRF® (Homogeneous Time-Resolved Fluorescence) technology. The cell line was grown in DMEM, GlutaMAX (Gibco # 61965), 10% (v/v) Foetal Bovine Serum (FBS), 0.5 mg/mL Geneticin, 100 units/ml Penicillin, 100 µg/ml Streptomycin, 1 mM Sodium Pyruvate, and 1x MEM non-essential
15 amino acids (Gibco # 11140) and seeded at 40,000 cells/well in poly-L-lysine-coated 96 well plates (Corning #354516) the day prior to assay.

On the day of analysis, growth medium was removed, and the cells were washed once with 150 µL Assay Buffer (0.05% v/v alkali-treated Casein (Sigma # C4765) and 100 µM
20 IBMX in Tyrodes Buffer (Tyrode's Salts (9.6 g/l), 10 mM HEPES, pH 7.4)) prior to compound incubation (15 min, 37°C) by addition of increasing concentrations of 1X final test compound (100 µL) in Assay Buffer.

The Assay Buffer was then removed, and the cells lysed (10 min, RT) in 80 µL Lysis
25 buffer, pH 7.4 (0.1 % w/v BSA, 5 mM HEPES, 0.3 % v/v Tween-20, 100 µM IBMX) per well. From each well, 10 µL cell lysate was transferred to a 384-well plate and mixed with 10 µL HTRF reagents diluted (1:40) in the kit provided lysis buffer. The plate was briefly shaken on a plate shaker (600 rpm, 1 min, RT) prior to incubation in the dark (1 hr, RT) before the response (TR-FRET ratio) was quantified using an Envision™ plate
30 reader (Perkin-Elmer).

Data from test compounds eliciting an increase in the intracellular level of cAMP upon GLP-2 R activation were normalized relative to the positive (teduglutide (GLP-2 analogue)), 1 nM) and negative control (vehicle) to calculate the EC₅₀ from the
35 concentration response curve using the 4-parameter logistic (4PL) nonlinear concentration response model based on the formula $Y = \text{Bottom} + (\text{Top} - \text{Bottom}) / (1 + 10^{((\text{LogEC}_{50} - X) * \text{HillSlope}))}$, where Y is percent activation, X is

compound concentrations and Top, Bottom, Hill Slope, and EC₅₀ are parameters fitted. The EC₅₀ value is a measure of the concentration of an agonist required to achieve half of that compound's maximal activation of the human GLP-2 receptor (hGLP-2R) activity in the assay.

5

Table 2: Potency of major degradation products and IsoAsp33 metabolite and isoAsp impurities in the hGLP2 R cAMP assay. EC50 is the geometric mean of the EC50 in nanomolar (nM) with 95% confidence interval (CI95) in brackets.

Drug substance and degradation products	Sequence	EC50_nM (CI95)
ZP1848 Drug Substance	H-HGEGTFSSELATILDALAARDFIAWLIATKITDKKKKKK-NH2 (SEQ ID NO: 1)	0.17 (0.13-0.22)
Kyn(25) (ZP8291)	H-HGEGTFSSELATILDALAARDFIAKynLIATKITDKKKKKK-NH2 (SEQ ID NO: 14)	0.64 (0.39-1.1)
Des-His(1) (ZP3915)	H-GEGTFSSELATILDALAARDFIAWLIATKITDKKKKKK-NH2 (SEQ ID NO: 15)	8.3 (5.8-12)
Des-His(1)Gly(2) (ZP3913)	H-EGTFSSELATILDALAARDFIAWLIATKITDKKKKKK-NH2 (SEQ ID NO: 16)	9.0 (6.3-13)
isoAsp(33) (ZP6885)	H-HGEGTFSSELATILDALAARDFIAWLIATKIT[isoAsp]-KKKKKK-NH2 (SEQ ID NO: 4)	0.054 (0.047-0.063)
isoAsp(33) metabolite (ZP11896)	H-HGEGTFSSELATILDALAARDFIAWLIATKIT[isoAsp]K-OH (SEQ ID NO: 6)	0.047 (0.036-0.061)
ZP1848 metabolite M1 (ZP2711)	H-HGEGTFSSELATILDALAARDFIAWLIATKITDKK-OH (SEQ ID NO: 2)	0.072 (0.049-0.11)
ZP1848 metabolite M2 (ZP2469)	H-HGEGTFSSELATILDALAARDFIAWLIATKITDK-OH (SEQ ID NO: 3)	0.043 (0.030-0.063)

Iso-Asp(15) (ZP2531)	H- HGEGTFSSELATIL[isoAsp]ALAARDFIAWLIATKITDKKKKKK- NH2 (SEQ ID NO: 17)	1300 (1163-1437)
Iso-Asp(21) (ZP4201)	H- HGEGTFSSELATILDALAAR[isoAsp]FIAWLIATKITDKKKKKK- NH2 (SEQ ID NO: 18)	110 (61-159)

Results

The potency data in Table 2 shows that isoAsp(33) and ZP1848 metabolites M1 and M2 are approximately 3 times more potent than ZP1848 in the hGLP-2 R cAMP assay performed. The ZP1848 isoAsp(33) metabolite has similarly potency to isoAsp(33), whereas the other major degradation products have reduced potencies compared to ZP1848.

Conclusion

As isoAsp(33) is a degradation product of ZP1848 with a significant potency activity on the GLP-2 receptor and therefore is contributing to the activity of the pharmacodynamic effects of glepaglutide drug product on the GLP-2 receptor, it is defined as a related substance of ZP1848.

Example 3: Calculation/prediction of shelf-life of the ZP1848 drug product

The shelf-life of the ZP1848 drug product is predicted from physical and chemical parameters which are generally subject to change during the shelf life of a drug product under the above given storage condition. Shelf-life is defined as the time period over which the drug product will be within the predefined acceptance criteria for specified parameters tested. The period starts from the manufacturing date of the drug product. Acceptance criteria are based on evaluation of batch data relating to stability.

As described above, the ZP1848 drug product was analyzed by the UHPLC analytical procedure for the determination of degradation products and content. The ZP1848 drug product process capability and stability evaluation of representative batches of the drug product has been used to set the specification limits of content.

In the present context, the term “content” refers to the amount of ZP1848 and related substances as measured in a chemical analysis (for example by HPLC) and expressed as percentage of the drug product label (% label claim). In contrast, the “amount” or “potency” relates to the activity of the pharmacodynamic effects of glepaglutide drug

product (i.e. ZP1848 and related substances) on the GLP-2 receptor, for example as determined in a cell based assay such as the one used in Example 2. To accommodate the degradation observed during storage, a lower shelf-life acceptance criterion of assay of not less than 90% of label claim is in general accepted by the health authorities.

5

The potency data in Table 2 shows that the degradation product isoAsp(33) is equally or more potent than the main compound ZP1848 and the metabolite of isoAsp(33) is equally potent to isoAsp(33). IsoAsp(33) is therefore considered as a related substance and is contributing to the activity of the pharmacodynamic effects of the drug product on the GLP-2 receptor. Accordingly, it is justified that the content of isoAsp(33) may be added to the content of the ZP1848 in the calculation of the total content in the ZP1848 drug product.

10

Accordingly, isoAsp(33) can be included in the content calculation (% label claim) using the following formula:

15

$$\text{Content (\% label claim)} = \frac{\text{Content ZP1848} \left(\frac{\text{mg}}{\text{mL}} \right) + \text{Content isoAsp(33)} \left(\frac{\text{mg}}{\text{mL}} \right)}{\text{label claim} \frac{\text{mg}}{\text{mL}}} \times 100$$

When isoAsp(33) is included in the content, its contribution to the total content increases over time as ZP1848 decreases and the content of isoAsp(33) increases over time. This has the benefit of extending the shelf-life of the formulation significantly because the drop in content is slower and it takes thus longer time for the drug product to reach the typical specification limit for drug content, i.e. 90% lower acceptance limit of content at end of shelf-life. An example of recalculation of the peptide content for one batch is shown in Table 3.

20

25

Table 3: Example of increase in content in a batch when isoAsp(33) is included in the reported content together with ZP1848 during storage at 2-8°C.

Time point/month	Total impurities %, isoAsp(33) included	Change in content when isoAsp(33) is included in content % of label claim (absolute)
0	1.4	+0.3
12	3.1	+1.4
18	4.2	+2.1
24	5.2	+2.6
30	6.2	+3.3
36	7.0	+3.5

As shown in Table 3, isoAsp(33) contributes to the drug content of the formulations, determined as % of label claim with estimated 1.4% of the content after 12 months, 2.1% of the content after 18 months 2.6% of the content after 24 months, 3.3% of the content after 30 months, and/or 3.5% of the content after 36 months at 2-8°C (absolute values).

- 5 This in turn means that the shelf life of aqueous formulations of ZP1848 may be extended significantly.

Table 4: Example of increase in content in a batch when isoAsp(33) is included in the reported content together with ZP1848 during storage at 25°C.

10

Time point/month	Total impurities %, isoAsp(33) included	Change in content when isoAsp(33) is included in content % of label claim (absolute)
0	1.4	+0.3
1	3.6	+1.8
3	7.8	+4.6
6	13.4	+8.4

As shown in Table 4, the amount of total impurities increase faster under accelerated conditions at 25°C compared to conditions under 2-8°C, and the relative increase in content when isoAsp(33) is included - compared to when isoAsp(33) is not included - becomes more significant during a shorter storage time. This implies that it is possible due to the activity of the isoAsp(33) degradation product to allow the drug product first to be stored at 2 to 8°C for a certain number of months and then be kept for a certain number of days or weeks at room temperature 20°C, 25°C or 30°C. This storage at room temperature (consecutive storage or dual storage or in-use period or additional storage condition) has the advantage that patients are granted more flexible storage and handling conditions when using the drug or drug product, because they can leave the drug product at room temperature for a certain number of days or weeks (e.g. when travelling) without affecting the safety, efficacy and/or quality of the drug product.

25

Table 5: Examples of shelf life in months with an allowed degradation in % when content is calculated as ZP1848 or ZP1848 plus isoAsp(33). Shelf life is calculated from slope of content during storage at 2-8°C:

Allowed degradation during shelf life	1%	2%	3%	4%	5%	6%	7%
Shelf life, months (ZP1848)	5	11	16	21	27	32	37
Shelf life, months (ZP1848 + IsoAsp(33) ZP1848)	11	22	32	43	54	65	76
Difference in shelf life, months*	5	11	16	22	27	33	38

* The difference is calculated on unrounded values.

The data in Table 5 shows that the inclusion of the IsoAsp(33) degradation product in the content determination leads to an additional 5 to 27 months of shelf life of the ZP1848 drug product compared with a ZP1848 drug product which does not have IsoAsp(33) included in the content of the product.

As an example, Figure 1 illustrates the time to reach 4% of loss in content (data shown in Table 5) when ZP1848/glepaglutide drug product is stored at 2-8°C. As the slope is less steep when content is calculated as ZP1848/glepaglutide and isoAsp(33) compared to ZP1848/glepaglutide, the time to reach 4% of loss in content is extended from 21 to 43 months. Thus, shelf-life is extended by 22 months if allowed degradation, given as 4% loss of content as an example.

Example 4: *In vivo* activity of ZP6885 H-HGEGTFSSELATILDALAARDFIAWLIATKIT-[Iso-Asp]-KKKKKK-NH₂ (SEQ ID NO: 4) on the intestinal growth effects of compounds in C57BL mice

The purpose of this study was to demonstrate the ability of the present compounds to stimulate small intestinal growth in C57BL (male) mice. Compounds ZP1848 and ZP1848 isoAsp(33) were administered by subcutaneous injection q.d for 10 days to C57BL mice at a dose of 30 nmol/kg (n=9/dose group).

Results are shown in Figure 2 and demonstrate that at equimolar doses compound ZP1848 and compound ZP1848 isoAsp(33) causes significant growth stimulation of the small intestine compared to vehicle treatment. No difference between compound ZP1848 and compound ZP1848 isoAsp(33) on small intestinal growth, measured as wet weight in mg of empty small intestine, was observed.

Example 5: Characterization of peak with relative retention time 1.15 in UHPLC chromatogram corresponding to isoAsp(33) ZP1848 by LC-MS

Figure 5 shows the chromatogram of aged ZP1848/glepaglutide formulation and the degradation peak with relative retention time 1.15. The identification of the peak in the chromatogram of ZP1848/glepaglutide drug product corresponding to RRT 1.15 was confirmed by LC-MS analysis of degraded drug product samples where a mass equal to lepaglutide was detected (4313.43 Da) and charge state [+3] in the peak RRT 1.15) (Figures 3 and 4).

Example 6: Confirmation of impurity assignment by spiking of synthesised impurities

Based on applicant's experience with peptide degradation products, most peptides with masses different than the parent peptide can be identified by LC-MS and/or spiking with a synthesized impurity (see Table 6). The degradation product in the peak at RRT 1.15 of the degraded drug product has the same mass as ZP1848 (LC-MS). Looking at the peptide sequence for ZP1848, the potential isomers are racemized isomers (racemization of individual amino acids) and isomerization of aspartate residues and glutamate residues. Degraded ZP1848 formulations have been tested for chiral purity and these data support that the racemization in ZP1848 is low and racemization cannot explain the observed increase in RRT 1.15. The potential isomers of the aspartate residues (Asp15, Asp21 and Asp33) and the glutamate residues (Glu3 and Glu9) have been synthesized and spiked into ZP1848 samples to establish the relative retention time by UHPLC. The only peak matching RRT 1.15 is IsoAsp33. Therefore, by the principles of elimination, it is possible to conclude that the degradation product increasing in RRT 1.15 is isoAsp(33).

Table 6 - Synthesized impurities with the same mass as ZP1848/glepaglutide

Impurity name designation	Relative retention time
IsoAsp(15)	0.65
IsoGlu(9)	0.72
Glepaglutide (ZP1848)	1.00
IsoGlu(3)	1.02
IsoAsp(33)	1.15
IsoAsp(21)	1.19

Figure 6 shows examples of spiking of synthesized impurities into drug product and injection into UHPLC (upper chromatogram) and lower chromatogram shows the elution of individual synthesized isoAsp(33) injected on the UHPLC.

5 **Example 7: Direct evidence that the peak with a relative retention time 1.15 in aged glepoglutide /ZP1848 formulation is isoAsp(33) by isolation and degradation by Asp-N enzyme**

To confirm a direct evidence of the degradation peak eluting at a relative retention time of 1.15 in the UHPLC chromatogram, an experiment which purifies the peak by collecting
10 the fraction corresponding to the peak can be conducted.

Asp-N enzyme, which cleaves peptides specifically on the N-terminal side of Aspartate (Asp) and cysteic acid residues, can be used to show the presence of IsoAsp residues in peptide sequences. ZP1848 contains three aspartate residues: Asp at position 15, at
15 position 21 and at position 33. The Asp-N enzyme will not cleave Iso-Asp residues. ZP1848 does not contain cysteic acid residues.

Asp-N enzyme is added to the isolated sample and incubated. The incubated sample is analysed by MS.
20

A sample of synthesized isoAsp(33) ZP1848 undergoes same treatment with Asp-N enzyme as described above.

The MS spectra of purified isoAsp(33) and synthesized isoAsp(33) would show the same
25 peptide sequence fractions (mass) corresponding to Asp-N enzyme cleavage of position 15 and 21 in the sequence, but no cleavage in position 33 indicating iso-Asp at the position. Thus, the experiment confirms that purified peak is identical to synthesized isoAsp(33) ZP1848.

30 While the present invention has been described in conjunction with the embodiments described above, many equivalent modifications and variations will be apparent to those skilled in the art when given this disclosure. Accordingly, the embodiments of the invention set forth are considered to be illustrative and not limiting. Various changes to the described embodiments may be made without departing from the spirit and scope of
35 the invention. All documents cited herein are expressly incorporated by reference in their entirety for all purposes.

Table 7Peptide concentration (mg/mL)	90% of column 1 ^b	90% of column 2 [[Lowest value of ZP1848 during the shelf life of the product]] ^a	10% of column 2 [[Highest value of isoAsp(33) ZP1848 during shelf life of the product]] ^a	Ranges for beginning of shelf life product [ZP1848]/[isoAsp(33) ZP1848]	Ranges for middle of shelf life product [ZP1848]/[isoAsp(33) ZP1848]	Ranges for end of shelf life product [ZP1848]/[isoAsp(33) ZP1848]
2	1.8	1.62	0.18	[1.99-1.85]/[0.01-0.05]	[1.90-1.75]/[0.05-0.15]	[1.85-1.62]/[0.10-0.18]
5	4.5	4.05	0.45	[4.99-4.60]/[0.01-0.15]	[4.70-4.25]/[0.10-0.35]	[4.30-4.05]/[0.25-0.45]
10	9	8.1	0.9	[9.99-9.20]/[0.01-0.55]	[9.30-8.60]/[0.35-0.75]	[8.70-8.10]/[0.50-0.9]
20	18	16.2	1.8	[19.99-18.20]/[0.01-0.90]	[18.50-17.30]/[0.75-1.10]	[17.50-16.20]/[0.90-1.8]

55

- a. These calculations are based upon about 6-8 months storage at 25°C (Table 4) or about 54 months storage at 2-8°C (Table 3 – extrapolated) - based on the amount of ZP1848 conversion to isoAsp(33) ZP1848 during the life of the product.
- b. 90% activity at the end of shelf life.

Claims:

1. A GLP-2 analogue is represented by the formula:
- H-HGEGTFSSELATILDALAARDFIWLIATKIT-[Iso-Asp]-KKKKKK-NH₂ (SEQ ID NO: 4);
- 5 or a pharmaceutically acceptable salt thereof, for use in therapy.
2. A stable liquid pharmaceutical formulation for use in therapy, the formulation comprising as active ingredients a first glucagon-like peptide 2 (GLP-2) analogue, wherein the first GLP-2 analogue is represented by the formula:
- 10 H-HGEGTFSSELATILDALAARDFIWLIATKIT-[Iso-Asp]-KKKKKK-NH₂ (SEQ ID NO: 4); or a pharmaceutically acceptable salt thereof; and
- 15 a second glucagon-like peptide 2 (GLP-2) analogue, wherein the second GLP-2 analogue is represented by the formula:
- H-HGEGTFSSELATILDALAARDFIWLIATKITDKKKKK-NH₂ (SEQ ID NO: 1);
- 20 or a pharmaceutically acceptable salt thereof.
3. A stable liquid pharmaceutical formulation for use in therapy, the formulation comprising as an active ingredient a glucagon-like peptide 2 (GLP-2) analogue, wherein the GLP-2 analogue is represented by the formula:
- 25 H-HGEGTFSSELATILDALAARDFIWLIATKIT-[Iso-Asp]-KKKKKK-NH₂ (SEQ ID NO: 4);
- or a pharmaceutically acceptable salt thereof, wherein the formulation comprises a GLP-2 analogue at a concentration of about 2 mg/mL to about 30 mg/ml, a buffer selected from the group consisting of a histidine buffer, mesylate buffer and acetate buffer, the buffer being present at a concentration of about 5 mM to about 50 mM, a non-ionic
- 30 tonicity modifier selected from the group consisting of mannitol, sucrose, glycerol and sorbitol at a concentration of about 90 mM to about 360 mM, arginine q.s. to provide a pH of about 6.6 to about 7.4.
- 35 4. A stable liquid pharmaceutical formulation for use in a method for the treatment and/or prevention of a stomach and bowel-related disorder in a human patient, the

formulation comprising as an active ingredient a glucagon-like peptide 2 (GLP-2) analogue, wherein the GLP-2 analogue is represented by the formula:

H-HGEGTFSSELATILDALAARDFIAWLIATKIT-[Iso-Asp]-KKKKKK-NH₂ (SEQ ID NO: 4)

5

or a pharmaceutically acceptable salt thereof, wherein the formulation comprises a GLP-2 analogue at a concentration of about 2 mg/mL to about 30 mg/ml, a buffer selected from the group consisting of a histidine buffer, mesylate buffer and acetate buffer, the buffer being present at a concentration of about 5 mM to about 50 mM, a non-ionic
10 tonicity modifier selected from the group consisting of mannitol, sucrose, glycerol and sorbitol at a concentration of about 90 mM to about 360 mM, arginine q.s. to provide a pH of about 6.6 to about 7.4.

5. The GLP-2 analogue or formulation according to any one of the preceding claims,
15 wherein a total drug substance content of the GLP-2 analogue present in a formulation is determined by adding a percentage content of the GLP-2 analogue present in the formulation to a percentage of the corresponding isoAsp(33) isomerisation product of the GLP-2 analogue to provide the total drug substance content.

20 6. The GLP-2 analogue or formulation according to any one of the preceding claims, wherein the formulation is a ready-to-use formulation.

7. The GLP-2 analogue or formulation according to any one of the preceding claims,
 wherein the GLP-2 analogue is present in the formulation at a concentration of about 15
25 mg/mL to about 25 mg/ml.

8. The GLP-2 analogue or formulation according to any one of the preceding claims,
 wherein the GLP-2 analogue is present in the formulation at a concentration of about 2
30 mg/mL.

9. The GLP-2 analogue or formulation according to any one of claim 1 to 8, wherein the GLP-2 analogue is present in the formulation at a concentration of about 5 mg/mL.

10. The GLP-2 analogue or formulation according to any one of claim 1 to 8, wherein
35 the GLP-2 analogue is present in the formulation at a concentration of about 10 mg/mL.

11. The GLP-2 analogue or formulation according to any one of claim 1 to 8, wherein

the GLP-2 analogue is present in the formulation at a concentration of about 20 mg/mL.

12. The GLP-2 analogue or formulation according to any one of the preceding claims, wherein the formulation consists of the GLP-2 analogue at a concentration of about 2
5 mg/mL to about 30 mg/mL, a buffer selected from the group consisting of a histidine buffer, mesylate buffer and acetate buffer, the buffer being present at a concentration of about 5 mM to about 50 mM, a non-ionic tonicity modifier selected from the group consisting of mannitol, sucrose, glycerol and sorbitol at a concentration of about 90 mM to about 360 mM, arginine q.s. to provide a pH of about 6.6 to about 7.4.
10
13. The GLP-2 analogue or formulation according to any one of claims 1 to 9, wherein the formulation comprises the GLP-2 analogue at a concentration of about 20 mg/mL, histidine buffer at a concentration of about 15 mM, mannitol at a concentration of about 230 mM, and arginine q.s. to provide a pH of about 7.0.
15
14. The GLP-2 analogue or formulation according to any one of claims 1 to 9, wherein the formulation comprises the GLP-2 analogue at a concentration of about 5 mg/mL or 10 mg/mL, histidine buffer at a concentration of about 15 mM, mannitol at a concentration of about 230-260 mM, and 4 mM to 5 mM arginine to provide a pH of
20 about 7.0.
15. The GLP-2 analogue or formulation according to any one of the preceding claims, wherein the formulation is stable at 2-8°C for at least 6 months, at least 10 months, at least 12 months, at least 18 months, at least 24 months, at least 30 months, or at least
25 36 months.
16. The GLP-2 analogue or formulation according to any one of the preceding claims, wherein the formulation is stable at 2-8°C for at least 6 months, at least 10 month, at least 12 months, at least 18 months, at least 24 months, at least 30 months, or at least
30 36 months and at least 14 days, at least one week or at least two weeks at room temperature of around 20°C, 25°C or 30°C.
17. The GLP-2 analogue or formulation according to claim 16 or claim 17, wherein the GLP-2 analogue in the formulation retains at least about 90% of its biological activity
35 after 18 months of storage 2-8°C.
18. The GLP-2 analogue or formulation according to any one of the preceding claims,

wherein the formulation is for administration to a subject by injection, and optionally by subcutaneous injection.

19. The GLP-2 analogue or formulation according to any one of the preceding claims,
5 wherein the liquid aqueous formulation, after having been stored for a certain number of months at 2 to 8°C, may then be kept for a certain number of days or weeks at room temperatures between 20°C to 30°C, preferably at room temperatures between 20°C to 25°C, more preferably at room temperatures between 25°C to 30°C.

10 20. The GLP-2 analogue or formulation according to any one of the preceding claims for use in a method for the treatment and/or prevention of a stomach and bowel-related disorder in a human patient, wherein the stomach and bowel-related disorder is ulcers, digestion disorders, malabsorption syndromes, short-gut syndrome, cul-de-sac
15 syndrome, inflammatory bowel disease, celiac sprue (for example arising from gluten induced enteropathy or celiac disease), tropical sprue, hypogammaglobulinemic sprue, enteritis, regional enteritis (Crohn's disease), ulcerative colitis, small intestine damage or short bowel syndrome (SBS).

21. The formulation of the glucagon-like peptide 2 (GLP-2) analogue for use in the
20 method of treatment and/or prevention of claim 20, wherein the stomach and bowel-related disorder is short bowel syndrome or ulcerative colitis.

22. The formulation of the glucagon-like peptide 2 (GLP-2) analogue for use in a
method for the treatment according to claim 20, wherein the stomach and bowel-related
25 disorder is radiation enteritis, infectious or post-infectious enteritis, or small intestinal damage due to toxic or other chemotherapeutic agents.

23. A method of treating a stomach or bowel related disorder in a subject in need
thereof, the method comprising administering to the subject stable liquid pharmaceutical
30 formulation comprising 1.9 ± 0.1 mg/mL of a mixture of active glucagon-like peptide 2 (GLP-2) analogues selected from:

(i) a first GLP-2 analogue having the formula H-
HGEFTFSSELATILDALAARDFIATKIT-[Iso-Asp]-KKKKKK-NH₂ (SEQ ID NO: 4), or
35 a pharmaceutically acceptable salt thereof, in an amount of from 0.01 to 0.18 mg/mL;
and

(ii) a second GLP-2 analogue having the formula H-HGEGTFSSELATILDALAARDFIAWLIATKITDKKKKKK-NH₂ (SEQ ID NO: 1), or a pharmaceutically acceptable salt thereof, in an amount of from 1.62 to 1.99 mg/mL.

5 24. The method of claim 23, wherein the formulation comprises the first GLP-2 analogue in an amount of 0.01 to 0.05 mg/mL and the second GLP-2 analogue in an amount of 1.85 to 1.99 mg/mL.

25. The method of claim 23, wherein the formulation comprises the first GLP-2
10 analogue in an amount of 0.05 to 0.15 mg/mL and the second GLP-2 analogue in an amount of 1.75 to 1.90 mg/mL.

26. The method of claim 23, wherein the formulation comprises the first GLP-2
15 analogue in an amount of 0.10 to 0.18 mg/mL and the second GLP-2 analogue in an amount of 1.62 to 1.85 mg/mL.

27. A method of treating a stomach or bowel related disorder in a subject in need thereof, the method comprising administering to the subject stable liquid pharmaceutical formulation comprising 4.7 ± 0.5 mg/mL of a mixture of active glucagon-like peptide 2
20 (GLP-2) analogues selected from:

(i) a first GLP-2 analogue having the formula H-HGEGTFSSELATILDALAARDFIAWLIATKIT-[Iso-Asp]-KKKKKKK-NH₂ (SEQ ID NO: 4), or a pharmaceutically acceptable salt thereof, in an amount of from 0.01 to 0.45 mg/mL; and

25 (ii) a second GLP-2 analogue having the formula H-HGEGTFSSELATILDALAARDFIAWLIATKITDKKKKKK-NH₂ (SEQ ID NO: 1), or a pharmaceutically acceptable salt thereof, in an amount of from 4.05 to 4.99 mg/mL.

28. The method of claim 27, wherein the formulation comprises the first GLP-2
30 analogue in an amount of 0.01 to 0.15 mg/mL and the second GLP-2 analogue in an amount of 4.60 to 4.99 mg/mL.

29. The method of claim 28, wherein the formulation comprises the first GLP-2
35 analogue in an amount of 0.10 to 0.35 mg/mL and the second GLP-2 analogue in an amount of 4.25 to 4.70 mg/mL.

30. The method of claim 28, wherein the formulation comprises the first GLP-2 analogue in an amount of 0.25 to 0.45 mg/mL and the second GLP-2 analogue in an amount of 4.05 to 4.30 mg/mL.
- 5 31. A method of treating a stomach or bowel related disorder in a subject in need thereof, the method comprising administering to the subject stable liquid pharmaceutical formulation comprising 9.30 ± 1 mg/mL of a mixture of active glucagon-like peptide 2 (GLP-2) analogues selected from:
- (i) a first GLP-2 analogue having the formula H-
10 HEGTFSSELATILDALAARDFIAWLIATKIT-[Iso-Asp]-KKKKKK-NH₂ (SEQ ID NO: 4), or a pharmaceutically acceptable salt thereof, in an amount of from 0.01 to 0.9 mg/mL; and
- (ii) a second GLP-2 analogue having the formula H-
HEGTFSSELATILDALAARDFIAWLIATKITDKKKKK-NH₂ (SEQ ID NO: 1), or a
pharmaceutically acceptable salt thereof, in an amount of from 8.10 to 9.99 mg/mL.
- 15 32. The method of claim 31, wherein the formulation comprises the first GLP-2 analogue in an amount of 0.01 to 0.55 mg/mL and the second GLP-2 analogue in an amount of 9.20 to 9.99 mg/mL.
- 20 33. The method of claim 31, wherein the formulation comprises the first GLP-2 analogue in an amount of 0.35 to 0.75 mg/mL and the second GLP-2 analogue in an amount of 8.60 to 9.30 mg/mL.
34. The method of claim 31, wherein the formulation comprises the first GLP-2
25 analogue in an amount of 0.50 to 0.90 mg/mL and the second GLP-2 analogue in an amount of 8.10 to 8.70 mg/mL.
35. A method of treating a stomach or bowel related disorder in a subject in need thereof, the method comprising administering to the subject stable liquid pharmaceutical
30 formulation comprising 18.5 ± 2 mg/mL of a mixture of active glucagon-like peptide 2 (GLP-2) analogues selected from:
- (i) a first GLP-2 analogue having the formula H-
HEGTFSSELATILDALAARDFIAWLIATKIT-[Iso-Asp]-KKKKKK-NH₂ (SEQ ID NO: 4), or
a pharmaceutically acceptable salt thereof, in an amount of from 0.01 to 1.8 mg/mL; and
- 35 (ii) a second GLP-2 analogue having the formula H-
HEGTFSSELATILDALAARDFIAWLIATKITDKKKKK-NH₂ (SEQ ID NO: 1), or a
pharmaceutically acceptable salt thereof, in an amount of from 16.20 to 19.99 mg/mL.

36. The method of claim 35, wherein the formulation comprises the first GLP-2 analogue in an amount of 0.01 to 0.90 mg/mL and the second GLP-2 analogue in an amount of 18.20 to 19.99 mg/mL.

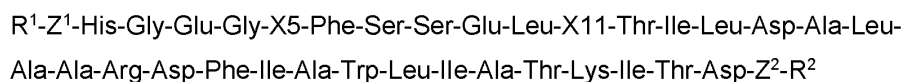
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37. The method of claim 35, wherein the formulation comprises the first GLP-2 analogue in an amount of 0.75 to 1.10 mg/mL and the second GLP-2 analogue in an amount of 17.30 to 18.50 mg/mL.

10 38. The method of claim 35, wherein the formulation comprises the first GLP-2 analogue in an amount of 0.90 to 1.80 mg/mL and the second GLP-2 analogue in an amount of 16.20 to 17.50 mg/mL.

15 39. A delivery device containing the GLP-2 analogue or formulation as defined in any one of claims 1 to 22, optionally wherein the delivery device is pre-filled syringe, an injector device, an injector pen, an adjustable dose auto-injector, a disposable auto-injector, a wearable injector, an infusion pump.

20 40. A method of assaying a formulation comprising a glucagon-like peptide 2 (GLP-2) analogue to determine a total drug substance content of the formulation, wherein the GLP-2 analogue is represented by the formula:



25

wherein:

R¹ is hydrogen, C₁₋₄ alkyl (e.g. methyl), acetyl, formyl, benzoyl or trifluoroacetyl;

X5 is Ser or Thr;

X11 is Ala or Ser;

30 R² is NH₂ or OH; and

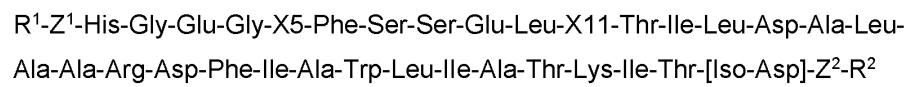
Z¹ and Z² are independently absent or a peptide sequence of 1-6 amino acid units of Lys;

or a pharmaceutically acceptable salt thereof; wherein the method comprises:

35 determining an amount of the GLP-2 analogue present in the formulation;

determining an amount of a corresponding isoAsp33 degradation product of the GLP-2

analogue represented by the formula:



5

and adding the amounts determined in steps (a) and (b) to provide the total substance content of the GLP-2 analogue present in the formulation.