

PHARMACEUTICAL FORMULATIONS OF AN AMYLIN RECEPTOR AGONIST
AND A GLP-1 RECEPTOR AGONIST COMPRISING A CYCLODEXTRIN

TECHNICAL FIELD

The current invention relates to a pharmaceutical formulation which is a co-
5 formulation of a GLP-1 receptor agonist and an amylin receptor agonist. Said pharmaceutical
formulation may be used for the medical treatment of subjects with overweight or obesity,
with or without one or more associated co-morbidities; diabetes, with or without one or more
associated comorbidities; and/or one or more cardiovascular diseases.

BACKGROUND

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Semaglutide is a glucagon-like peptide 1 (GLP-1) receptor agonist and is the active
pharmaceutical ingredient in Ozempic®. Ozempic® is indicated (i) as an adjunct to diet and
exercise to improve glycemic control in adults with type 2 diabetes mellitus and (ii) to reduce
the risk of major adverse cardiovascular events in adults with type 2 diabetes mellitus and
15 established cardiovascular disease.

Semaglutide is also the active pharmaceutical ingredient in Wegovy®. Wegovy® is
indicated as an adjunct to a reduced calorie diet and increased physical activity for chronic
weight management in adult patients with an initial body mass index (BMI) of greater or
equal to 30 kg/m² or greater than 27 kg/m², in the presence of at least one weight-related co-
20 morbidity.

Ozempic® and Wegovy® are liquid pharmaceutical formulations comprising 8 mM
phosphate and having a pH of about 7.4.

A fixed-dose combination of an amylin receptor agonist, cagrilintide, and the GLP-1
receptor agonist, semaglutide, has been investigated for the treatment of overweight and
25 obesity (Lancet 2021; 397: 1736–48). The drug products investigated were in the form of
separate liquid pharmaceutical formulations for subcutaneous use, comprising either
cagrilintide or semaglutide.

Thus far, it has not been considered possible to co-formulate semaglutide and
cagrilintide, due to the different physicochemical properties of these active pharmaceutical
30 ingredients. Semaglutide, a GLP-1 receptor agonist, has an isoelectric point that is
incompatible with the optimal pH of cagrilintide, an amylin receptor agonist. Semaglutide is
optimally stable at pH 7.4 and has previously been formulated in a neutral to slightly basic
solution of pH 7-8, to ensure an acceptable chemical and physical stability in aqueous

solution. Cagrilintide is optimally stable at pH 4.0 and has been formulated in acidic solution, as an increasing pH accelerates the rate of its chemical degradation. The different physicochemical properties of cagrilintide and semaglutide preclude a simple mixture of these two peptides. The same applies to other GLP-1 receptor agonist and amylin receptor agonist combinations when the two have incompatible optimal pH ranges.

There remains a need in the art for a simple means of co-administering a GLP-1 receptor agonist such as semaglutide and an amylin receptor agonist such as cagrilintide.

There remains a similar need in the art for such means to be suitable for multiple use.

10 SUMMARY OF THE INVENTION

Disclosed herein is a means of co-formulating an amylin receptor agonist and a GLP-1 receptor agonist. Disclosed herein is a pharmaceutical formulation comprising an amylin receptor agonist, a GLP-1 receptor agonist, a cyclodextrin comprising hydrophilic chemical substitutions such as hydroxypropyl substitutions and at least one preservative and/or stabilising agent. The cyclodextrin may be of the hydroxypropyl-substituted alpha type, comprising six ring-arranged glucose units. The cyclodextrin may be of the hydroxypropyl-substituted beta type, comprising seven ring-arranged glucose units. The at least one preservative may be m-cresol and/or phenol and/or EDTA. The at least one stabilising agent may be EDTA. The pharmaceutical formulation may further comprise a buffer, such as histidine or citrate; a tonicity agent, such as sorbitol and/or propylene glycol; and/or a surfactant such as polysorbate 20 and/or 80. The pharmaceutical formulation may be a liquid formulation. The liquid pharmaceutical formulation has a pH within the range of 5.6-6.4, preferably 5.8-6.2. The pharmaceutical formulation disclosed herein may be administered by parenteral injection, preferably subcutaneous injection. The pharmaceutical formulation disclosed herein may be suitable for multiple use.

The pharmaceutical formulation of the current invention may be multiply advantageous. It enables co-formulation of an amylin receptor agonist and a GLP-1 receptor agonist, is suitable for multiple use in that it demonstrates efficacy against microbial growth and is well tolerated when injected subcutaneously.

The pharmaceutical formulation disclosed herein may be used for the medical treatment of subjects with overweight or obesity, with or without one or more associated co-morbidities and/or diabetes, with or without one or more associated co-morbidities. The

pharmaceutical formulation disclosed herein may improve convenience, treatment compliance and ultimately improved clinical outcome in such patients.

DESCRIPTION

5 Disclosed herein is a pharmaceutical formulation comprising an amylin receptor agonist, a GLP-1 receptor agonist, a cyclodextrin comprising hydroxypropyl substitutions and at least one preservative and/or stabilising agent. Disclosed herein is a liquid pharmaceutical formulation comprising an amylin receptor agonist, a GLP-1 receptor agonist, a cyclodextrin comprising hydroxypropyl substitutions and at least one preservative and/or stabilising agent.

10 Disclosed herein is a means of co-formulating an amylin receptor agonist and a GLP-1 receptor agonist, wherein the GLP-1 receptor agonist has an isoelectric point that precludes its co-formulation in the pH range enabling chemical stability of the amylin receptor agonist. Disclosed herein is a means of co-formulating a GLP-1 receptor agonist having an isoelectric point (pI) of less than about 5.0, such as 3.0-5.0, such as 3.8-4.9, such as less
15 than about 4.5, such as 3.0-4.5, such as 3.5-4.5, such as 4.0-4.5; and an amylin receptor agonist.

The optimal pH of the amylin receptor agonist is the pH at which it is, chemically and physically, most stable. The person skilled in the art can easily find the amylin receptor agonist's optimal pH by testing its chemical and physical stability in an aqueous solution
20 essentially consisting of the amylin receptor agonist, a buffer and water for injection, across the pH range.

The optimal pH of the GLP-1 receptor agonist is the pH at which it is, chemically and physically, most stable. The person skilled in the art can easily find the GLP-1 receptor agonist's optimal pH by testing its chemical and physical stability, in an aqueous solution
25 essentially consisting of the GLP-1 receptor agonist, a buffer and water for injection, across the pH range. The physical stability of the GLP-1 receptor agonist may reflect its isoelectric point, which may coincide with the pH where poorest physical stability might be expected.

As will be apparent to the person skilled in the art, the chemical stability and purity of any GLP-1 receptor agonist and/or any amylin receptor agonist in any liquid formulation can
30 be quantified by means of, e.g., reversed phase (ultra) high performance liquid chromatography (RP-UHPLC or RP-HPLC) and/or by measuring the percentage of high molecular weight protein (%HMWP) by means of, e.g., size exclusion chromatography (SEC).

As will be apparent to the person skilled in the art, the physical stability of a GLP-1 receptor agonist and/or any amylin receptor agonist in any liquid formulation can be quantified by measuring particle formation and/or fibrillation by means of micro-flow imaging (MFI) or a Thioflavin T (ThT) fluorescence stress assay, respectively.

5 Disclosed here is a means of formulating an amylin receptor agonist and a GLP-1 receptor agonist whose optimal pHs differ by at least about two pH units, such as 2-5 pH units, such as 2-4 pH units, such as 3-5 pH units.

The GLP-1 receptor agonist may have an isoelectric point (pI) of less than 5.0 or less than 4.5, such as 3.0-5.0, such as 3.5-5.0, such as 3.5-4.5, such as 3.8-4.9, such as 10 4.0-4.5. The GLP-1 receptor agonist may be semaglutide. The GLP-1 receptor agonist may be tirzepatide.

The amylin receptor agonist may have an isoelectric point (pI) of 7.6-9.4 or 8-9. The amylin receptor agonist may be a human amylin-derived peptide derivative having an isoelectric point (pI) of 7.6-9.4 or 8-9. The amylin receptor agonist may be cagrilintide, or a 15 biologically active metabolite or degradation product of cagrilintide. The amylin receptor agonist may be a peptide derivative disclosed in WO2013/156594, such as that of Example 52.

The composition of the formulation disclosed herein preserves and/or improves the chemical and physical stability of the active pharmaceutical ingredients, even when co- 20 formulated at pH 5.6-6.4; preserves the pharmacokinetic profiles of the active pharmaceutical ingredients in terms of their bioavailability and exposure; and exhibits an acceptable local tolerance upon subcutaneous injection.

The terms "pharmaceutical formulation", "co-formulation" and "drug product" may herein be used interchangeably to refer to a liquid pharmaceutical formulation comprising a 25 GLP-1 receptor agonist and an amylin receptor agonist.

The pharmaceutical formulation disclosed herein is suitable for parenteral injection, preferably subcutaneous injection.

The pharmaceutical formulation disclosed herein may be suitable for multiple use.

Amylin

30 The term "amylin" herein refers to a polypeptide having the same amino acid sequence as an endogenous amylin, such as human amylin.

Amylin receptor

An amylin receptor agonist may bind to and activate the calcitonin receptor (CTR) and/or the amylin receptors (AMYRs). The latter consist of heterodimers of two components: the calcitonin receptor (CTR) and one of three receptor activity-modifying proteins (RAMP1–3) resulting in three possible complexes, AMYR1–3.

Amylin receptor agonists

The pharmaceutical formulations disclosed herein comprise an amylin receptor agonist. An “amylin receptor agonist” may be defined as a chemical entity which is capable of binding to an amylin receptor and is capable of activating or “agonising” it. In the context of the current invention, the amylin receptor agonist is capable of binding to and activating at least the amylin receptor 3 (AMYR3). The amylin receptor agonist may also be capable of agonising the calcitonin receptor, the amylin receptor 1 (AMYR1) and/or the amylin receptor 2 (AMYR2).

Examples of endogenous amylin receptor agonists are human amylin and human calcitonin. Examples of exogenous amylin receptor agonists are cagrilintide and pramlintide (the active pharmaceutical ingredient in Symlin®).

The concentration of the amylin receptor agonist in the pharmaceutical formulation disclosed herein may be at least about 0.25 mg/ml. The concentration of the amylin receptor agonist in the pharmaceutical formulation disclosed herein may be, at the most, about 22 mg/ml. The concentration of the amylin receptor agonist in the pharmaceutical formulation disclosed herein may be such as to provide any one of the doses specified herein.

The amylin receptor agonist is capable of activating the amylin receptor; in other words, it is “potent” on the amylin receptor. The *in vitro* potency of the amylin receptor agonist on amylin receptor 3 may be measured as described in WO2022/129526, Assay 2. The potency of the compound may be described by means of its EC₅₀ value, wherein EC₅₀ represents the concentration of compound upon which 50% of its maximal effect is observed. The lower the EC₅₀ value, the more potent the compound.

When tested as described as described in WO2022/129526, Assay 2, the amylin receptor agonist as disclosed herein may have an EC₅₀ value of less than 300 pM, such as less than 200 pM, such as less than 150 pM, preferably less than 100 pM, such as less than 75 pM, preferably less than 50 pM, such as less than 40 pM, such as less than 30 pM, such as less than 20 pM, such as less than 10 pM.

Cagrilintide

The amylin receptor agonist in the pharmaceutical formulation disclosed herein may be cagrilintide or a biologically active metabolite or degradation product of cagrilintide.

Cagrilintide, also known as AM833, is the compound of Example 53 in
5 WO2012/168432: N-alpha-[(S)-4-Carboxy-4-(19-carboxynonadecanoylamino)butyryl]-
[Glu14,Arg17,Pro37]-pramlintide. Cagrilintide may be prepared as described in
WO2012/168432, pages 153-155.

Cagrilintide may be in the form of a salt, preferably a pharmaceutically acceptable salt.

10 A biologically active metabolite or degradation product of cagrilintide may have an aspartate (Asp) in position 21 or 22. A biologically active metabolite or degradation product of cagrilintide may have an iso-aspartate (iso-Asp) in position 21 or 22.

When the potency of cagrilintide was tested using the procedure described in
WO2022129526, Assay 2, cagrilintide had an EC₅₀ value of about 11 pM (WO2022/129526,
15 Tables 4b and 4c).

The concentration of cagrilintide in the pharmaceutical formulation disclosed herein may be from about 0.25 mg/ml to about 22 mg/ml.

The pharmaceutical formulation disclosed herein may comprise cagrilintide in a concentration of about 0.33-18 mg/ml; such as 0.25-0.5 mg/ml, such as about 0.33 mg/ml;
20 such as 0.5-1.0 mg/ml, such as about 0.67 mg/ml; such as 1.0-1.5 mg/ml, such as about 1.33 mg/ml; such as 1.5-2.0 mg/ml, such as about 1.5 mg/ml; such as 2.0-2.5 mg/ml; such as 2.5-3.0 mg/ml; such as 3.0-3.5 mg/ml; such as about 3.2 mg/ml; such as 3.5-4.0 mg/ml; such as 4.0-5.0 mg/ml; such as 5.0-6.0 mg/ml; such as 6.0-7.0 mg/ml, such as 7.0-8.0 mg/ml, such as 8.0-9.0 mg/ml, such as 9.0-10.0 mg/ml, such as about 9.6 mg/ml; such as 10-11
25 mg/ml, such as 11.0-12.0 mg/ml, such as 11-13 mg/ml; such as 13-22 mg/ml, such as about 18 mg/ml; such as about 20-22 mg/ml.

The concentration of cagrilintide in the pharmaceutical formulation disclosed herein may be at least about 0.25 mg/ml. The pharmaceutical formulation disclosed herein may comprise no more than 22 mg/ml cagrilintide. The pharmaceutical formulation disclosed
30 herein may comprise no more than 12 mg/ml cagrilintide.

GLP-1

The term "GLP-1" or "native GLP-1" herein refers to human Glucagon-Like Peptide-1 (GLP-1(7-37)).

GLP-1 receptor agonist

The pharmaceutical formulations disclosed herein comprise a GLP-1 receptor agonist. A "GLP-1 receptor agonist" may be defined as a ligand which is capable of binding to the GLP-1 receptor and producing a biological response similar to that of the endogenous ligand, glucagon-like peptide 1 (GLP-1(7-37)). A "full" GLP-1 receptor agonist may be defined as a GLP-1 receptor agonist which is capable of eliciting a biological response of the same magnitude as GLP-1(7-37).

Examples of known exogenous GLP-1 receptor agonists include exenatide (the active pharmaceutical ingredient in Byetta®), liraglutide (the active pharmaceutical ingredient in Victoza® and Saxenda®, first disclosed in WO98/08871, Example 37), lixisenatide (the active pharmaceutical ingredient in Lyxumia®, disclosed in WO01/04156), retatrutide (disclosed in WO2019/125938, Example 12), semaglutide (the active pharmaceutical ingredient in Ozempic®, Rybelsus® and Wegovy®), and tirzepatide (the active pharmaceutical ingredient in Mounjaro®/Zepbound®, disclosed in WO2016/111971, Example 1, and in U.S. Patent No. 9,474,780).

The GLP-1 receptor agonist may be a GLP-1(7-37) peptide derivative, such as semaglutide or liraglutide.

The GLP-1 receptor agonist may be within a compound that also agonises one or more other receptors; such as a dual- or triple- receptor agonist, such as tirzepatide. The GLP-1 receptor agonist may be tirzepatide.

The concentration of the GLP-1 receptor agonist in the pharmaceutical formulation disclosed herein may be at least about 0.25 mg/ml. The concentration of the GLP-1 receptor agonist in the pharmaceutical formulation disclosed herein may be, at the most, about 30 mg/ml. The concentration of the GLP-1 receptor agonist in the pharmaceutical formulation disclosed herein may be from about 0.25 mg/ml to about 30 mg/ml. The concentration of the GLP-1 receptor agonist in the pharmaceutical formulation disclosed herein may be from about 0.25 mg/ml to about 22 mg/ml. The concentration of the GLP-1 receptor agonist in the pharmaceutical formulation disclosed herein may be from about 5 mg/ml to about 30 mg/ml. The concentration of the GLP-1 receptor agonist in the pharmaceutical formulation disclosed herein may be such as to provide any one of the doses specified herein.

The GLP-1 receptor agonist is capable of binding to and activating, or "agonising" the GLP-1 receptor; in other words, it is "potent" on the GLP-1 receptor. The *in vitro* potency of the GLP-1 receptor agonist on the GLP-1 receptor may be measured as described in WO/2022/129526, Assay 1. The potency of the compound may be described by means of its

EC₅₀ values, wherein EC₅₀ represents the concentration of compound upon which 50% of its maximal effect is observed. The lower the EC₅₀ value, the more potent the compound.

When tested as described in WO/2022/129526, Assay 1, the GLP-1 receptor agonist disclosed herein may have an EC₅₀ value of less than 300 pM, such as less than 200 pM,
5 such as less than 150 pM, preferably less than 100 pM, such as less than 75 pM, even more preferably less than 50 pM, such as less than 40 pM, such as less than 30 pM, such as less than 20 pM, such as less than 10 pM.

Semaglutide

Semaglutide is a GLP-1 receptor agonist also known as N^{6,26}-{18-[N-(17-
10 carboxyheptadecanoyl)-L-γ-glutamyl]-10-oxo-3,6,12,15-tetraoxa-9,18-diazaoctadecanoyl]-[8-(2-amino-2-propanoic acid),34-L-arginine]human glucagon-like peptide 1(7-37)}. Semaglutide was described in WO2006/097537 and in *J. Med. Chem.* 2015, 58, 18, 7370–7380 and may be manufactured using methods well known to the person skilled in the art, such as that briefly described in WO2006/097537, Example 4.

15 Semaglutide may be present in the current pharmaceutical formulation in its fully or partly ionised form; for example one or more carboxylic acid groups (-COOH) may be deprotonated into the carboxylate group (-COO⁻) and/or one or more amino groups (-NH₂) may be protonated into the -NH₃⁺ group.

Semaglutide may be in the form of a salt, preferably a pharmaceutically acceptable
20 salt.

When the potency of semaglutide was tested according to the procedure described in WO/2022/129526, Assay 1, semaglutide had an EC₅₀ value of about 5.5 pM (see WO/2022/129526, Tables 4b and 4c).

The concentration of semaglutide in the pharmaceutical formulation disclosed herein
25 may be from about 0.25 mg/ml to about 22 mg/ml.

The pharmaceutical formulation may comprise semaglutide in a concentration of about 0.33-18 mg/ml; such as 0.25-0.5 mg/ml, such as about 0.33 mg/ml; such as 0.5-1.0 mg/ml, such as about 0.67 mg/ml; such as 1.0-1.5 mg/ml, such as about 1.33 mg/ml; such as 1.5-2.0 mg/ml, such as about 1.5 mg/ml; such as 2.0-2.5 mg/ml; such as about 2.2 mg/ml,
30 such as 2.5-3.0 mg/ml; such as 3.0-3.5 mg/ml; such as about 3.2 mg/ml; such as 3.5-4.0 mg/ml; such as 4.0-5.0 mg/ml; such as about 4.8 mg/ml; such as 5.0-6.0 mg/ml; such as 6.0-7.0 mg/ml, such as about 6.4 mg/ml; such as 7.0-8.0 mg/ml, such as about 8.0 mg/ml; such as 8.0-9.0 mg/ml, such as 9.0-10.0 mg/ml, such as about 9.6 mg/ml; such as 10-11 mg/ml, such as about 10.7 mg/ml; such as 11.0-12.0 mg/ml, such as 11-13 mg/ml; such as about

12.8 mg/ml; such as 13-22 mg/ml, such as about 16 mg/ml; such as about 18 mg/ml; such as about 20-22 mg/ml.

The concentration of semaglutide in the pharmaceutical formulation disclosed herein may be at least about 0.25 mg/ml. The pharmaceutical formulation disclosed herein may
5 comprise no more than 22 mg/ml semaglutide. The pharmaceutical formulation disclosed herein may comprise no more than 12 mg/ml semaglutide.

Methods of manufacture

The GLP-1 receptor agonist and/or amylin receptor agonist in the pharmaceutical formulation disclosed herein may, for instance, be produced by classical peptide synthesis,
10 e.g. solid phase peptide synthesis using t-Boc or Fmoc chemistry, or other well established techniques, such as those described in Greene and Wuts, "Protective Groups in Organic Synthesis", John Wiley & Sons, 1999; Florencio Zaragoza Dörwald, "Organic Synthesis on Solid Phase", Wiley-VCH Verlag GmbH, 2000; and "Fmoc Solid Phase Peptide Synthesis", Edited by W.C. Chan and P.D. White, Oxford University Press, 2000.

15 Alternatively, the GLP-1 receptor agonist and/or amylin receptor agonist may be produced by recombinant expression techniques, e.g. by culturing a host cell containing a DNA sequence encoding the peptide sequence and capable of expressing the peptide, in a suitable nutrient medium under conditions permitting the expression of the peptide. Non-limiting examples of host cells suitable for expression of these peptides are *Escherichia coli*,
20 *Saccharomyces cerevisiae* and mammalian BHK or CHO cell lines.

GLP-1 receptor agonists comprising one or more non-proteogenic amino acids may also be produced, semi-synthetically, using a combination of recombinant expression techniques and chemical peptide synthesis as described in WO2009/083549. Compounds which comprise one or more non-natural amino acids and/or a covalently attached N-terminal
25 mono- or dipeptide mimetic may also be produced as described in Hodgson et al in "The synthesis of peptides and proteins containing non-natural amino acids", *Chemical Society Reviews*, vol. 33, no. 7 (2004), p. 422-430.

Once the GLP-1 receptor agonist and amylin receptor agonist (drug substances) have been manufactured and purified, the pharmaceutical formulation (drug product)
30 disclosed herein may be prepared using the method described in WO2023/187067.

Alternatively, or subsequently, the composition comprising the drug substances and cyclodextrin, such as the drug product, may be freeze- or spray-dried using methods known to the person skilled in the art. Ohtake, S., Izutsu, K. I., & Lechuga-Ballesteros, D. (Eds.). describe such methods in "*Drying technologies for biotechnology and pharmaceutical*

applications" (2020) John Wiley & Sons. The composition comprising the drug substances and cyclodextrin that is dried may further comprise a surfactant, such as polysorbate 20 and/or 80.

If the composition is intermediately freeze- or spray-dried then the dry formulation is dissolved or "reconstituted" in aqueous solution prior to use. Said aqueous solution may be presented in a vial. Said aqueous solution may comprise or consist of a pre-determined amount of water for injection. Said aqueous solution may comprise or consist of a pre-determined amount of water for injection and one or more preservatives. Said aqueous solution may comprise or consist of a pre-determined amount of water for injection and phenol and/or m-cresol and/or EDTA. Said aqueous solution may comprise or consist of a pre-determined amount of water for injection, phenol and/or EDTA. Said aqueous solution may comprise or consist of a pre-determined amount of water for injection and m-cresol and/or EDTA. Said aqueous solution may comprise or consist of a pre-determined amount of water for injection and phenol and/or m-cresol. Said aqueous solution may comprise or consist of a pre-determined amount of water for injection and phenol. Said aqueous solution may comprise or consist of a pre-determined amount of water for injection and m-cresol. Said aqueous solution may comprise or consist of a pre-determined amount of water for injection and EDTA. Said aqueous solution may further comprise a buffer having at least one pKa value of about 5.0-7.0, such as histidine or citrate. Said aqueous solution may further comprise sorbitol.

Whether or not the formulation is intermediately freeze- or spray-dried, the liquid pharmaceutical formulation ultimately administered to the patient has the composition disclosed herein.

Isoelectric point

The isoelectric point (pI) of a molecule is the pH at which a molecule carries no net charge. The pI of a peptide, such as the peptide backbone of the GLP-1 receptor agonist or the peptide backbone of the amylin receptor agonist, may be theoretically calculated from the pK values of the peptide's amino acids and of the terminal amine and carboxyl groups and can be used to predict the solubility of the peptide at a given pH.

The overall net charge of a protein or peptide is related to the solution pH and can be approximated using the Henderson-Hasselbalch equation (Po HN, Senozan NM. The Henderson-Hasselbalch Equation: Its History and Limitations. J Chem Educ. 2001;78(11):1499). To derive the isoelectric point or find the charge of a peptide at a specific pH, the pKa values of its amino acids are considered. At pH 7.0, the carboxyl group is

negative (-1) and the amino group is positive (+1). The net charge of a peptide at a given pH is the sum of the charges of the ionizable groups in the peptide at the given pH. By summing the charges of all ionizable groups, the overall charge of the peptide can be determined.

Several software tools exist that can predict the charge states and aid in understanding a peptide's behavior under different pH conditions [IPC - Isoelectric Point Calculator, Kozlowski, Lukasz P., Biology Direct (2016), 11, 55/1-55/16]. Because algorithms that consider the amino acid pKa values are utilized, predictions are not considered to diverge significantly [Principles of Biochemistry, A.L. Lehninger and M. M. Cox, 1982].

When chemical modifications are made to the peptide, these may affect the pI; however, by evaluating the pKa for the ionizable groups for the modifications, these can be accounted for in the pI prediction also by using the Henderson-Hasselback equation. An example of such "chemical modification" is a protraction moiety, such as a side chain comprising a fatty acid, that is covalently bound to the peptide.

The charge of a peptide influences its solubility. Peptides with a net charge, whether positive or negative, have enhanced solubility in water because the charged groups can interact with water molecules [Pace CN, Grimsley GR, Scholtz JM. Protein ionizable groups: pK values and their contribution to protein stability and solubility. J Biol. Chem. 2009;284(20):13285–13289], [The effect of net charge on the solubility, activity, and stability of ribonuclease Sa, Shaw, Kevin L.; Grimsley, Gerald R.; Yakovlev, Gennady I.; Makarov, Alexander A.; Pace, C. Nick, Protein Science (2001), 10 (6), 1206-1215]. Thus, good solubility is typically obtained at a formulation pH away from the isoelectric point where the peptide carries sufficient charge, positive or negative, to accommodate solubility.

In the context of the current invention, the theoretically calculated or predicted isoelectric point of the GLP-1 receptor agonist may be equal to or less than about 5.0, preferably less than about 4.5. The theoretically calculated/predicted isoelectric point of the GLP-1 receptor agonist may be in the range of 3.5-5.0, such as 3.8-4.9, such as 3.5-4.5, such as about 4.0-4.5. For example, semaglutide has a theoretically calculated/predicted isoelectric point of about 4.37. Liraglutide has a theoretically calculated/predicted isoelectric point of about 4.47. Tirzepatide has a theoretically calculated/predicted isoelectric point of about 4.03. Retatrutide has a theoretically calculated/predicted isoelectric point of about 3.93.

There are other GLP-1 receptor agonists having theoretically calculated isoelectric points above 4.5.

The theoretically calculated isoelectric point of the amylin receptor agonist may have an isoelectric point (pI) in the range of 7.6-9.4 or 8-9. Cagrilintide has a theoretically calculated isoelectric point of about 8.56.

There are other amylin receptor agonists having theoretically calculated isoelectric points above 9.4.

Excipients

Cyclodextrin

The pharmaceutical formulation disclosed herein comprises a cyclodextrin comprising hydroxypropyl substitutions.

10 The pharmaceutical formulation may comprise more than 10% w/v of a cyclodextrin comprising hydroxypropyl substitutions. The pharmaceutical formulation may comprise less than 22% w/v of a cyclodextrin comprising hydroxypropyl substitutions. The pharmaceutical formulation may comprise about 10-20% w/v, about 12-18% w/v, about 10-17.5% w/v, about 11.25-15%, such as about 15% w/v of a cyclodextrin comprising hydroxypropyl substitutions.

15 Cyclodextrins are oligosaccharide starch derivatives consisting of 6, 7 or 8 α -(1,4)-linked glucopyranose (glucose) units arranged cyclically and denoted the alpha, beta or gamma type, respectively. The cyclodextrins have a wide range of applications, amongst others as pharmaceutical excipients [P. Breen & S. S. Jambhekar, Cyclodextrins in pharmaceutical formulations II: solubilization, binding constant, and complexation efficiency, 20 Drug Discovery Today, Volume 21, Number 2 February 2016]. Guidelines on their use as pharmaceutical excipients have been described by the European Medicines Agency [Background review for cyclodextrins used as excipients, 2014, EMA/CHMP/333892/2013, Committee for Human Medicinal Products (CHMP)], [Cyclodextrins used as excipients, 2017, EMA/CHMP/333892/2013, Committee for Human Medicinal Products (CHMP)]. Cyclodextrin 25 types that do not carry hydrophilic substitutions have poor solubility and are rarely used for parenteral drug products.

In order to improve the solubility of cyclodextrins, the hydroxyl groups of the glucose units of the cyclodextrins may be substituted by a varying number of hydrophilic chemical substitutions e.g. by hydroxypropyl groups, leading to differences in degree of substitution 30 which can be described as either the average number of hydroxypropyl per cyclodextrin molecule (abbreviated DS) or the molar substitution degree corresponding to the average number of hydroxypropyl per glucose units present in the cyclodextrin in question (abbreviated MS). The value of hydroxypropyl per cyclodextrin can be achieved by

multiplication of the molar substitution degree by the number of glucose units comprised in the cyclodextrin in question. Difference in degree of substitution can result in alterations in physicochemical properties such as surface activity and complexing abilities. The hydroxyl groups may also be chemically substituted by groups of sulfobutylether. These mostly hydrophilic modifications have yielded cyclodextrin derivatives highly suitable for parenteral administration [Cyclodextrins used as excipients, 2017, EMA/CHMP/333892/2013, Committee for Human Medicinal Products (CHMP)]. Cyclodextrins comprising hydroxypropyl substitutions are commonly abbreviated HP-CDs whereas cyclodextrins comprising sulfobutylether substitutions are abbreviated SBE-CDs.

The cyclodextrins comprising hydrophilic substitutions adopt what may be described as cone-like shapes having a hydrophobic inner cavity and a hydrophilic outer surface formed by the many hydrophilic substitutions capable of forming hydrogen bonds with neighbouring water molecules, thereby improving water solubility [T. Loftsson, Cyclodextrins in Parenteral Formulations, Journal of Pharmaceutical Sciences, 2020, 1-11].

Their hydrophobic microenvironment inside the cavity of these cone-like structures enables them to form drug-to-cyclodextrin complexes mainly through hydrophobic interactions [T. Loftsson, Cyclodextrins in Parenteral Formulations, Journal of Pharmaceutical Sciences, 2020, 1-11]. As a complex is formed between cyclodextrin and a drug molecule carrying one or more hydrophobic regions, these as well as the hydrophobic cavity of cyclodextrin become shielded from water, thereby increasing the solubility of the complex compared to the solubility of the individual constituents. Also, once the complex between cyclodextrin and peptide molecules is formed, it impairs the intermolecular interactions that typically leads to aggregation [T. Loftsson, Cyclodextrins in Parenteral Formulations, Journal of Pharmaceutical Sciences, 2020, 1-11].

The pharmaceutical formulation disclosed herein preferably comprises a cyclodextrin of the hydroxypropyl-substituted alpha type and/or a cyclodextrin of the hydroxypropyl-substituted beta type.

Unexpectedly, such cyclodextrin carrying hydroxypropyl substitutions was found superior, in its ability to stabilise a co-formulation of cagrilintide and semaglutide, than the same cyclodextrin type carrying sulfobutylether substitutions.

The pharmaceutical formulation disclosed herein may comprise a cyclodextrin of the hydroxypropyl-substituted alpha type, comprising six ring-arranged glucose units. The hydroxypropyl substituted cyclodextrin of the alpha type is abbreviated HP-A-CD. Hydroxypropyl-alpha-cyclodextrins (CAS: 128446-33-3/99241-24-4) are commercially

available, with an average molar substitution (MS) of 0.8 and a molar substitution range of 0.5-0.9.

The pharmaceutical formulation disclosed herein may comprise hydroxypropyl-alpha-cyclodextrin having a minimum of about 0.4 hydroxypropyls per glucose unit. The pharmaceutical formulation disclosed herein may comprise hydroxypropyl-alpha-cyclodextrin having a maximum of about 1.2 hydroxypropyls per glucose unit.

The pharmaceutical formulation disclosed herein may comprise hydroxypropyl-alpha-cyclodextrin having a molar substitution range of 0.5-0.9 hydroxypropyls per glucose unit. The pharmaceutical formulation disclosed herein may comprise hydroxypropyl-alpha-cyclodextrin having an average molar substitution (MS) of about 0.8 hydroxypropyls per glucose unit.

The pharmaceutical formulation disclosed herein may comprise a cyclodextrin of the hydroxypropyl-substituted beta type, comprising seven ring-arranged glucose units. The hydroxypropyl substituted cyclodextrin of the beta type is abbreviated HP-B-CD.

Hydroxypropyl-beta-cyclodextrins are well known pharmaceutical excipients, typically used in small molecule pharmaceutical formulations, primarily to increase solubility and bioavailability [T. Loftsson, Cyclodextrins in Parenteral Formulations, Journal of Pharmaceutical Sciences, 2020, 1-11]. Thus far, the use of cyclodextrins and cyclodextrin substituted derivatives in protein and peptide-based pharmaceutical formulations is limited.

The commercially available hydroxypropyl substitution degrees (DS) for hydroxypropyl-beta-cyclodextrins as pharmaceutical excipients ranges between 2.8 and 10.5 according to the European and US pharmacopoeia [USP 38 NF 33, Pharm Eur 8, as estimated by methods described in USP <761> /Pharm. Eur. 2.2.33], corresponding to 0.4-1.5 hydroxypropyl per glucose unit (MS). Commercially available cyclodextrins such as hydroxypropyl-beta-cyclodextrins are usually described by means of the average molar substitutions (MS) of their molar substitution ranges.

Hydroxypropyl-beta-cyclodextrins (CAS: 128446-35-5/94035-02-6) are commercially available for use as excipients, with average molar substitutions (MS) including: MS 0.62, with a molar substitution range of 0.58-0.68; MS 0.67, with a molar substitution range from (0.6-0.9); MS 0.68, with a molar substitution range from (0.58-0.72); MS 0.84, with a molar substitution range from (0.8-1.0); MS 0.92, with a molar substitution range from (0.81-0.99); MS 1.08, with a molar substitution range from (0.86-1.14); each value describing the number of hydroxypropyls per glucose unit.

The pharmaceutical formulation disclosed herein may comprise hydroxypropyl-beta-cyclodextrin having a minimum of about 0.4 hydroxypropyls per glucose unit. The

pharmaceutical formulation disclosed herein may comprise hydroxypropyl-beta-cyclodextrin having a maximum of about 1.2 hydroxypropyls per glucose unit.

The pharmaceutical formulation disclosed herein may comprise hydroxypropyl-beta-cyclodextrin having a molar substitution range of 0.58-1.0 hydroxypropyls per glucose unit.

- 5 The pharmaceutical formulation disclosed herein may comprise hydroxypropyl-beta-cyclodextrin having an average molar substitution (MS) range of about 0.62-0.92 hydroxypropyls per glucose unit.

- 10 The pharmaceutical formulation disclosed herein may comprise hydroxypropyl-beta-cyclodextrin having an average molar substitution (MS) of about 0.62-0.84 hydroxypropyls per glucose unit.

The pharmaceutical formulation disclosed herein may comprise hydroxypropyl-beta-cyclodextrin having an average molar substitution (MS) of about 0.62. The pharmaceutical formulation disclosed herein may comprise hydroxypropyl-beta-cyclodextrin having about 0.58-0.68 hydroxypropyls per glucose unit.

- 15 The pharmaceutical formulation disclosed herein may comprise hydroxypropyl-beta-cyclodextrin having an average molar substitution (MS) of about 0.68. The pharmaceutical formulation disclosed herein may comprise hydroxypropyl-beta-cyclodextrin having about 0.58-0.72 hydroxypropyls per glucose unit.

- 20 The pharmaceutical formulation disclosed herein may comprise hydroxypropyl-beta-cyclodextrin having an average molar substitution (MS) of about 0.67. The pharmaceutical formulation disclosed herein may comprise hydroxypropyl-beta-cyclodextrin having about 0.6-0.9 hydroxypropyls per glucose unit.

- 25 The pharmaceutical formulation disclosed herein may comprise hydroxypropyl-beta-cyclodextrin having an average molar substitution (MS) of about 0.84. The pharmaceutical formulation disclosed herein may comprise hydroxypropyl-beta-cyclodextrin having about 0.8-1.0 hydroxypropyls per glucose unit.

- 30 The pharmaceutical formulation disclosed herein may comprise hydroxypropyl-beta-cyclodextrin having an average molar substitution (MS) of about 0.92. The pharmaceutical formulation disclosed herein may comprise hydroxypropyl-beta-cyclodextrin having about 0.81-0.99 hydroxypropyls per glucose unit.

- 35 The pharmaceutical formulation disclosed herein may comprise more than 10% w/v and less than 22% w/v, such as about 10-20% w/v, such as about 12-18% w/v, such as about 10-17.5% w/v, such as about 11.25-15%, such as about 15% w/v hydroxypropyl-beta-cyclodextrin having a minimum of about 0.4 hydroxypropyls per glucose unit and a maximum of about 1.2 hydroxypropyls per glucose unit; such as an average of 0.62-0.92

hydroxypropyls per glucose unit, such as about 0.75 hydroxypropyls per glucose unit; such as an average of 0.62-0.84 hydroxypropyls per glucose unit; such as an average of 0.62 hydroxypropyls per glucose unit, such as about 0.58-0.68 hydroxypropyls per glucose unit.

Cyclodextrins bind and form complexes with the hydrophobic moieties of molecules.

- 5 The interaction between several common pharmaceutical preservatives and cyclodextrin is well documented and represents a major obstacle in the development of preserved liquid pharmaceutical formulations comprising cyclodextrin (Loftsson, T., Stefansdottir, O., Friðriksdóttir, H., & Guðmundsson, Ö. (1992), *Drug development and industrial pharmacy*, 18(13), 1477-1484). First of all, when the preservative-cyclodextrin complex is formed, the
- 10 preservative loses its antimicrobial effect. Secondly, the preservative competes and displaces the drug in the drug-cyclodextrin complex, greatly reducing both the chemical and physical stability of the formulation. The pharmaceutical formulation disclosed herein has involved overcoming these well-known obstacles.

Other excipients

- 15 The pharmaceutical formulation disclosed herein may comprise one or more preservatives. Preservatives for use in pharmaceutical formulations are well-known to the skilled person. For convenience, reference is made to Remington: *The Science and Practice of Pharmacy*, 21st edition, 2006. The concentration of the one or more preservatives must be such that the regulatory requirements for a pharmaceutical formulation for multiple use with
- 20 respect to antimicrobial efficacy are fulfilled. In other words, the purpose of the one or more preservatives is to inhibit microbial growth in the pharmaceutical formulation once the latter is no longer in sterile packaging. The most widely used pharmaceutical antimicrobial preservatives are aromatic organic compounds, such as benzyl alcohol, phenol, m-cresol and parabens.

- 25 The one or more preservatives in the pharmaceutical formulation disclosed herein may be m-cresol and/or phenol and/or EDTA.

One preservative in the pharmaceutical formulation disclosed herein may be m-cresol in a concentration of 9-40 mM.

- 30 One preservative in the pharmaceutical formulation disclosed herein may be phenol in a concentration of 18-65 mM.

The preservatives in the pharmaceutical formulation disclosed herein may be m-cresol in a concentration of 9-40 mM and phenol in a concentration of 18-65 mM.

One preservative in the pharmaceutical formulation disclosed herein may be EDTA in a concentration of 0.5-5.0 mg/ml.

The preservatives in the pharmaceutical formulation disclosed herein may be m-cresol in a concentration of 9-40 mM, phenol in a concentration of 18-65 mM and EDTA in a concentration of 0.5-5.0 mg/ml.

5 The pharmaceutical formulation may comprise a stabilising agent. The use of a stabilising agent in pharmaceutical formulations is well-known to the skilled person. For convenience, reference is made to Remington: *The Science and Practice of Pharmacy*, 21st edition, 2006. The stabilising agent may be EDTA. The stabilising agent may be EDTA in a concentration of 0.5-5.0 mg/ml.

10 In the context of the formulation disclosed herein, EDTA may thus serve as a preservative and/or as a stabilising agent.

The pharmaceutical formulation may comprise a buffer. The use of a buffer in pharmaceutical formulations is well-known to the skilled person. For convenience, reference is made to Remington: *The Science and Practice of Pharmacy*, 21st edition, 2006.

15 pH may be measured at "room temperature", typically defined as 15-25°C or 15-20°C. pH is preferably measured at about 20°C.

The pharmaceutical formulation disclosed herein may comprise a buffer having a pKa close to the desired pH of the solution. The pharmaceutical formulation may comprise a buffer having at least one pKa value of about 5.0-7.0. The pharmaceutical formulation may comprise a buffer having a pKa of about 5.0-7.0. The pharmaceutical formulation may
20 comprise a buffer selected from the group consisting of histidine, citrate and/or phosphate. The buffer may be citrate, in a concentration of 3-30 mM. The buffer may be histidine, in a concentration of 3-30 mM. The buffer may be phosphate, in a concentration of 3-30 mM.

The pharmaceutical formulation may further comprise one or more agents for adjusting pH, such as NaOH and/or HCl.

25 The desired pH of the pharmaceutical formulation may be about 5.6-6.4, such as about 5.8-6.2. The pH may be about 5.6, such as about 5.7, such as about pH 5.8, such as about 5.9, such as about 6.0, such as about 6.1, such as about 6.2, such as about 6.3, such as about 6.4. The pH is preferably about 6.0.

30 The pharmaceutical formulation may comprise a tonicity agent. The use of a tonicity agent in pharmaceutical formulations is well-known to the skilled person. For convenience, reference is made to Remington: *The Science and Practice of Pharmacy*, 21st edition, 2006.

The purpose of the tonicity agent is to protect living tissue when the formulation is injected into the body. The tonicity agent may be selected from the group consisting of glycerol, mannitol, propylene glycol, sorbitol or trehalose, or a combination thereof. The

tonicity agent may be glycerol. The tonicity agent may be mannitol. The tonicity agent may be propylene glycol. The tonicity agent may be sorbitol. The tonicity agent may be trehalose.

The concentration of the tonicity agent is such as to render the formulation isotonic.

Where the tonicity agent is glycerol, it may be present in a concentration of 2.5-18 mg/ml.

- 5 Where the tonicity agent is mannitol, it may be present in a concentration of 16.5-37.5 mg/ml, such as about 20 mg/ml. Where the tonicity agent is propylene glycol, it may be present in a concentration of about 2-15 mg/ml. Where the tonicity agent is sorbitol, it may be present in a concentration of about 5-35 mg/ml; such as about 10-30 mg/ml; such as about 16-28 mg/ml, such as about 16.5-25 mg/ml, such as about 16-26 mg/ml; such as about 16-24 mg/ml; such as about 26 mg/ml, such as about 24 mg/ml, such as about 22 mg/ml, such as about 20 mg/ml, such as about 18 mg/ml, such as about 16 mg/ml, such as about 12 mg/ml. Where the tonicity agent is trehalose, it may be present in a concentration of 33-75 mg/ml, such as about 38 mg/ml.
- 10

- The pharmaceutical formulation may comprise a surfactant. The surfactant may further increase the physical stability and robustness of a formulation during its manufacture, storage and use as a medicament; for example, preserve the stability of a formulation when it is exposed to air inside a container. The use of surfactants in pharmaceutical formulations is well-known to the skilled person. For convenience, reference is made to Remington: *The Science and Practice of Pharmacy*, 21st edition, 2006.
- 15

- 20 The surfactant may be selected from the group consisting of polysorbate 20 and/or polysorbate 80. The surfactant may be polysorbate 20. The surfactant is preferably polysorbate 80.

The pharmaceutical formulation may comprise 0.01 mg/ml or more polysorbate 20 and up to 2.0, such as up to 1.5 mg/ml polysorbate 20.

- 25 The pharmaceutical formulation may comprise about 0.01-1.0 mg/ml polysorbate 20, such as about 0.05 mg/ml polysorbate 20.

When presented in a vial, the pharmaceutical formulation may comprise more than about 0.1 and less than about 0.2 mg/ml polysorbate 20.

- The pharmaceutical formulation may comprise 0.01 mg/ml or more polysorbate 80 and up to 2.0, such as up to 1.5 mg/ml polysorbate 80.
- 30

The pharmaceutical formulation may comprise 0.01-0.1 mg/ml polysorbate 80; preferably about 0.05 mg/ml polysorbate 80.

When presented in a vial, the pharmaceutical formulation may comprise more than about 0.1 and less than about 0.2 mg/ml polysorbate 80.

The pharmaceutical formulation may comprise water for injection (WFI). The pharmaceutical formulation may comprise more than 75% w/w water, such as 80% w/w water, such as about 85% w/w water, such as up to 90% w/w water.

Medical utility

5 The pharmaceutical formulations disclosed herein may be for medical use.

The pharmaceutical formulation disclosed herein may be administered by parenteral injection. The pharmaceutical formulation disclosed herein may be administered by subcutaneous injection.

10 The term "treatment", as used herein, refers to the medical therapy of any human or other vertebrate subject in need thereof. Said subject is expected to have undergone physical examination by a medical practitioner, or a veterinary medical practitioner, who has given a tentative or definitive diagnosis which would indicate that the use of said specific treatment is beneficial to the health of said human or other vertebrate. The timing and purpose of said treatment may vary from one individual to another, according to the status
15 quo of the subject's health. Thus, said treatment may be prophylactic (preventative), palliative, symptomatic and/or curative.

The pharmaceutical formulation disclosed herein may be administered to a human subject.

The pharmaceutical formulation disclosed herein may be used in:

20 (i) the prevention and/or treatment of any form of diabetes and associated symptoms, such as hyperglycaemia, type 2 diabetes, impaired glucose tolerance, type 1 diabetes, non-insulin dependent diabetes, maturity onset diabetes of the young (MODY), gestational diabetes and/or for the reduction of HbA1c;

25 (ii) the delaying or prevention of diabetic disease progression, such as progression in type 2 diabetes, delaying the progression of impaired glucose tolerance (IGT) to insulin-requiring type 2 diabetes and/or delaying the onset and/or progression of non-insulin requiring type 2 diabetes to insulin-requiring type 2 diabetes;

30 (iii) the prevention and/or treatment of certain eating disorders, overweight and/or obesity; e.g. by decreasing food intake, suppressing appetite, inducing satiety, reducing body weight; treating or preventing binge eating disorder, food cravings, bulimia nervosa and/or obesity induced by medication, such as an antipsychotic or a steroid; reducing gastric motility; and/or delaying gastric emptying;

 (iv) the prevention and/or treatment of cardiovascular disease, such as the delaying or reduction of the development of a major adverse cardiovascular event (MACE) selected

from the group consisting of cardiovascular death, non-fatal myocardial infarction, non-fatal stroke, revascularisation, hospitalisation for unstable angina pectoris, and hospitalisation for heart failure;

5 (v) the prevention and/or treatment of non-alcoholic fatty liver disease (NAFLD) and/or non-alcoholic steatohepatitis (NASH), otherwise known as metabolic dysfunction-associated steatohepatitis (MASH), and/or alcoholic liver disease (ALD);

(vi) the prevention and/or treatment of chronic kidney disease;

(vii) the prevention and/or treatment of obstructive sleep apnoea;

10 (viii) the prevention and/or treatment of cognitive disorders such as Alzheimer's disease.

The indication may be (i). The indication may be (ii). The indication may be (iii). The indication may be (iv). The indication may be (v). The indication may be (vi). The indication may be (vii). The indication may be (viii). The indication may be overweight or obesity. The indication may be type 2 diabetes.

15 Generally, all subjects suffering from obesity are also considered to be suffering from overweight. The subject suffering from obesity may be a human being, such as an adult human or a child, wherein "child" includes the infant and the adolescents.

The World Health Organisation (WHO) defines obesity as being the abnormal or excessive accumulation of fat that may impair health and considers body mass index (BMI) 20 to be the most convenient population-level measure of overweight and obesity. The formula used to calculate BMI is weight in kilograms (kg)/height in meters squared (m^2).

For adults, the WHO defines overweight and obesity as follows: overweight means having a BMI greater than or equal to 25; obesity means having a BMI greater than or equal to 30.

25 For children, the WHO considers age when defining overweight and obesity.

For children under the age of five, overweight means having a weight-for-height greater than two standard deviations above the WHO Child Growth Standards median; and obesity means having a weight-for-height greater than three standard deviations above the WHO Child Growth Standards median.

30 Overweight and obesity are defined as follows for children aged five to nineteen: overweight means having a BMI-for-age that is greater than one standard deviation above the WHO Growth Reference median; and obesity means having a BMI-for-age that is greater than two standard deviations above the WHO Growth Reference median.

Nonetheless, the diagnostic criteria for underweight, the normal range, pre-obesity/overweight and obesity can differ between countries/populations, as illustrated in Table (i) below for adults.

- 5 Table (i): Definitions of underweight, the normal range, pre-obesity/overweight and obesity in adults

Nutritional status	BMI (kg/m ²)			
	International/ European/ US classification	Chinese population	Japanese classification	Taiwanese classification
Underweight	<18.5	<18.5	<18.5	<18.5
Normal weight	18.5-24.9	≥ 18.5 and < 23	≥ 18.5 and < 25	≥ 18.5 and < 24
Pre-obesity/ Overweight	25.0-29.9	$24 \leq \text{BMI} < 28$		≥ 24 and < 27
Obesity	≥ 30	≥ 28	≥ 25	
Obesity class I	30.0-34.9		≥ 25 and < 30	$27 \leq$ and < 30
Obesity class II	35.0-39.9		≥ 30 and < 35	$30 \leq$ and < 35
Obesity class III	≥ 40		≥ 35 and < 40	≥ 35
Obesity class IV			≥ 40	

Guidelines for the Asian population were published by Misra A et al. J Assoc Physicians India. 2009; 57:163-70.

- 10 Guidelines for the Chinese population were issued in the 2006 edition of the *Guidelines for Prevention and Control of Overweight and Obesity in Chinese Adults*, compiled by the Chinese Working Group on Obesity.

Guidelines for the Japanese population were issued, in 2016, by the Japanese Society for the Study of Obesity (JASSO) in *Guidelines for the management of obesity disease*.

- 15 Guidelines for the Taiwanese population were issued by the Taiwanese government's Health Promotion Administration (HPA), Ministry of Health and Welfare in 2023, in the 2nd edition of its "*Evidence-Based Guideline on Adult Obesity Prevention and Management*".

- 20 An adult human subject suffering from obesity may thus have a BMI of 25 kg/m² or more, 27 kg/m² or more, 28 kg/m² or more or 30 kg/m² or more; this subject may also be referred to as being obese. The obesity may be class I, class II, class III or class IV obesity.

An adult human subject suffering from overweight may have a BMI of 24 kg/m² or more, 25 kg/m² or more, or 27 kg/m² or more. In some embodiments a human subject suffering from overweight has a BMI in the range of 24 to <27 kg/m², in the range of 24 to < 28 kg/m², in the range of 25 to <30 kg/m² or in the range of 27 to <30 kg/m².

5 A higher than normal BMI increases the risk of an individual developing any one of a wide range of other diseases or co-morbidities. The weight-related co-morbidity may be one, or a combination of any one of the diseases mentioned in (i), (ii), (iv), (v) and (vii), above.

 The pharmaceutical formulation disclosed herein may be for use in the treatment or prevention of overweight, wherein the patient may have at least one weight-related co-
10 morbidity. The pharmaceutical formulation disclosed herein may be for use in the treatment or prevention of obesity, wherein the patient may have at least one weight-related co-morbidity.

 The pharmaceutical formulation disclosed herein may be used as an adjunct to a reduced-calorie diet and increased physical activity for chronic weight management, in a
15 subject that is obese at the start of treatment.

 The pharmaceutical formulation disclosed herein may be used as an adjunct to a reduced-calorie diet and increased physical activity for chronic weight management in a subject that is overweight at the start of treatment and has at least one weight-related co-morbidity.

20 The pharmaceutical formulation disclosed herein may be used as an adjunct to a reduced-calorie diet and increased physical activity for chronic weight management in an adult human being with an initial body mass index (BMI) of 30 kg/m² or more, 28 kg/m² or more, 27 kg/m² or more or 25 kg/m² or more.

 The pharmaceutical formulation disclosed herein may be used as an adjunct to a
25 reduced-calorie diet and increased physical activity for chronic weight management in an adult human being that has an initial body mass index (BMI) of 25 kg/m² or more or 24 kg/m² or more and at least one weight-related co-morbidity.

 Administration of the pharmaceutical formulation disclosed herein may result in
30 >15% weight loss, such as >20% weight loss, such as >25% weight loss, such as >30% weight loss, such as about 15-40% weight loss, such as about 20-35% weight loss, such as about 25-30% weight loss, within 26 weeks of the start of treatment.

 Administration of the pharmaceutical formulation disclosed herein disclosed herein may result in >15% weight loss, such as >20% weight loss, such as >25% weight loss, such as >30% weight loss, such as about 15-40% weight loss, such as about 20-35% weight loss, such as about 25-30% weight loss, within 26 weeks of the start of treatment.
35

The pharmaceutical formulation disclosed herein may be for use in the treatment or prevention of diabetes, as described in (i) or (ii), above. The pharmaceutical formulation disclosed herein may be for use in the treatment or prevention of diabetes and at least one diabetes-related co-morbidity, such as (vi), above.

5 Administration of the pharmaceutical formulation disclosed herein may result in a higher HbA_{1c} reduction, in %-points, compared to that which results from treatment with either the GLP-1 receptor agonist as sole active ingredient or the amylin receptor agonist as the sole active ingredient.

Administration of the semaglutide and cagrilintide pharmaceutical formulations
10 disclosed herein may result in a higher HbA_{1c} reduction, in %-points, compared to that which results from treatment with either semaglutide as sole active ingredient or cagrilintide as sole active ingredient.

Dosages

The pharmaceutical formulation of the invention comprises a specific concentration
15 of amylin receptor agonist and a specific concentration of GLP-1 receptor agonist. For example, as mentioned above, the pharmaceutical formulation may comprise from 0.25 to 22 mg/ml cagrilintide and from 0.25 to 22 mg/ml semaglutide. The doses of GLP receptor agonist and amylin receptor agonist administered in a single injection depend on the concentrations of these active ingredients within the pharmaceutical formulation and the
20 volume of pharmaceutical formulation administered.

The pharmaceutical formulation of the invention may be administered as a single dose at predefined intervals.

A single dose of the pharmaceutical formulation disclosed herein may contain any one of the following doses of an amylin receptor agonist, such as cagrilintide, and a GLP-1
25 receptor agonist, such as semaglutide.

An effective amount of an amylin receptor agonist, such as cagrilintide, and a GLP-1 receptor agonist, such as semaglutide, may be administered to a subject in need thereof.

The dose may be administered approximately once weekly. The interval between two fixed doses may be about 4 days, about 5 days, about 6 days, about 7 days, about 8
30 days, about 9 days or about 10 days. In a preferred embodiment, a fixed maintenance dose is administered approximately once every 7 days (once weekly).

The dose may be administered to an individual having any one or a combination of the diseases or co-morbidities listed above. In some preferred embodiments, the dose is administered a subject with obesity (body mass index [BMI] ≥ 30 kg/m²). In some preferred

embodiments, the dose is administered to individuals that are overweight (BMI ≥ 27 kg/m²–
<30 kg/m²) and that have at least one weight-related co-morbidity (e.g. hypertension, type 2
diabetes mellitus, or dyslipidaemia).

5 In some embodiments, once weekly treatment results in a statistically significant,
dose-dependent, reduction in body weight.

In some preferred embodiments, the dose is administered as an adjunct to diet and
exercise to improve glycemic control in adults with type 2 diabetes mellitus.

10 Upon initiation of treatment, it may be beneficial to administer ascending doses of an
amylin receptor agonist, such as cagrilintide, and a GLP-1 receptor agonist, such as
semaglutide, to individuals in need thereof. Once the individual is acclimatised to the
treatment, it may be beneficial to administer maintenance doses of an amylin receptor
agonist such as cagrilintide and a GLP-1 receptor agonist such as semaglutide to individuals
in need thereof.

15 Treatment may be once weekly and the dose-escalation period may be 16 weeks.

Treatment may be once weekly, wherein dose escalation occurs approximately once
weekly.

Treatment may be once weekly, whereindose escalation occurs approximately once
every other week.

20 Treatment may be once weekly, whereindose escalation occurs approximately once
every three weeks.

Treatment may be once weekly, wherein dose escalation occurs approximately once
every four weeks.

The dose of amylin receptor agonist administered may be about 0.25-16 mg, such
as about 0.25-9.0 mg, such as about 0.25-4.5 mg, such as about 0.25-2.4 mg.

25 The dose of cagrilintide administered may be about 0.25-16 mg, such as about 0.25-
9.0 mg, such as about 0.25-4.5 mg, such as about 0.25-2.4 mg.

The dose of cagrilintide administered may be about 0.25 mg.

The dose of cagrilintide administered may be about 0.5 mg.

The dose of cagrilintide administered may be about 1.0 mg.

30 The dose of cagrilintide administered may be about 1.5 mg.

The dose of cagrilintide administered may be about 1.7 mg.

The dose of cagrilintide administered may be about 2.4 mg.

The dose of cagrilintide administered may be about 3.4 mg.

The dose of cagrilintide administered may be about 3.6 mg.

35 The dose of cagrilintide administered may be about 4.5 mg.

The dose of cagrilintide administered may be about 7.2 mg.

The dose of cagrilintide administered may be about 8.0 mg.

The dose of cagrilintide administered may be about 9.0 mg.

The dose of cagrilintide administered may be about 16.0 mg.

5 The dose of GLP-1 receptor agonist administered may be about 0.25-16 mg, such as about 0.25-9.0 mg, such as about 0.25-4.5 mg, such as about 0.25-2.4 mg.

 The dose of semaglutide administered may be about 0.25-16 mg, such as about 0.25-9.0 mg, such as about 0.25-4.5 mg, such as about 0.25-2.4 mg.

 The dose of semaglutide administered may be about 0.25 mg.

10 The dose of semaglutide administered may be about 0.5 mg.

 The dose of semaglutide administered may be about 1.0 mg.

 The dose of semaglutide administered may be about 1.5 mg.

 The dose of semaglutide administered may be about 1.7 mg.

 The dose of semaglutide administered may be about 2.4 mg.

15 The dose of semaglutide administered may be about 3.6 mg.

 The dose of semaglutide administered may be about 4.5 mg.

 The dose of semaglutide administered may be about 4.8 mg.

 The dose of semaglutide administered may be about 6.0 mg.

 The dose of semaglutide administered may be about 6.9 mg.

20 The dose of semaglutide administered may be about 7.2 mg.

 The dose of semaglutide administered may be about 8.0 mg.

 The dose of semaglutide administered may be about 9.0 mg.

 The dose of semaglutide administered may be about 12 mg.

25 The dose of semaglutide administered may be about 16.0 mg. The dose of semaglutide administered may be about 16.0 mg.

 The ratio of amylin receptor agonist to GLP-1 receptor agonist may be about 1:2.

 The ratio of cagrilintide to semaglutide may be about 1:2.

 The dose of cagrilintide may be about 0.125 mg and the dose of semaglutide may be about 0.25 mg.

30 The dose of cagrilintide may be about 0.25 mg and the dose of semaglutide may be about 0.5 mg.

 The dose of cagrilintide may be about 0.5 mg and the dose of semaglutide may be about 1.0 mg.

35 The dose of cagrilintide may be about 0.75 mg and the dose of semaglutide may be about 1.5 mg.

The dose of cagrilintide may be about 0.85 mg and the dose of semaglutide may be about 1.7 mg.

The dose of cagrilintide may be about 1.2 mg and the dose of semaglutide may be about 2.4 mg.

5 The dose of cagrilintide may be about 2.25 mg and the dose of semaglutide may be about 4.5 mg.

The dose of cagrilintide administered may be about 3.6 mg and the dose of semaglutide may be about 7.2 mg.

10 The dose of cagrilintide may be about 4.0 mg and the dose of semaglutide may be about 8.0 mg.

The dose of cagrilintide may be about 7.2 mg and the dose of semaglutide may be about 14.4 mg.

The dose of cagrilintide may be about 8.0 mg and the dose of semaglutide may be about 16.0 mg.

15 The maintenance dose of cagrilintide may be about 1.2 mg and the maintenance dose of semaglutide may be about 2.4 mg.

The maintenance dose of cagrilintide may be about 2.25 mg and the maintenance dose of semaglutide may be about 4.5 mg.

20 The maintenance dose of cagrilintide may be about 4.0 mg and the maintenance dose of semaglutide may be about 8.0 mg.

The maintenance dose of cagrilintide may be about 8.0 mg and the maintenance dose of semaglutide may be about 16.0 mg.

The ratio of amylin receptor agonist to GLP-1 receptor agonist may be about 1:1. The ratio of cagrilintide to semaglutide may be about 1:1.

25 The dose of cagrilintide may be about 0.25 mg and the dose of semaglutide may be about 0.25 mg.

The dose of cagrilintide may be about 0.5 mg and the dose of semaglutide may be about 0.5 mg.

30 The dose of cagrilintide may be about 1.0 mg and the dose of semaglutide may be about 1.0 mg.

The maintenance dose of cagrilintide may be about 1.0 mg and the maintenance dose of semaglutide may be about 1.0 mg. The dose of cagrilintide may be about 1.7 mg and the dose of semaglutide may be about 1.7 mg.

35 The maintenance dose of cagrilintide may be about 1.7 and the maintenance dose of semaglutide may be about 1.7.

The dose of cagrilintide may be about 2.4 mg and the dose of semaglutide may be about 2.4 mg.

The maintenance dose of cagrilintide may be about 2.4 mg and the maintenance dose of semaglutide may be about 2.4 mg.

5 The dose of cagrilintide may be about 4.5 mg and the dose of semaglutide may be about 4.5 mg.

The dose of cagrilintide may be about 8.0 mg and the dose of semaglutide may be about 8.0 mg.

10 The dose of cagrilintide may be about 16.0 mg and the dose of semaglutide may be about 16.0 mg.

The ratio of amylin receptor agonist to GLP-1 receptor agonist may be between 1:1 and 1:7.

The dose of cagrilintide may be about 2.4 mg and the dose of semaglutide may be about 2.4 mg to 16.0 mg.

15 The dose of cagrilintide may be about 2.4 mg and the dose of semaglutide may be about 3.6 mg to 16.0 mg.

The dose of cagrilintide may be about 2.4 mg and the dose of semaglutide may be about 2.4 mg to 13.5 mg.

20 The dose of cagrilintide may be about 2.4 mg and the dose of semaglutide may be about 3.6 mg to 13.5 mg.

The dose of cagrilintide may be about 2.4 mg and the dose of semaglutide may be about 3.6 mg.

The dose of cagrilintide may be about 2.4 mg and the dose of semaglutide may be about 4.8 mg.

25 The dose of cagrilintide may be about 2.4 mg and the dose of semaglutide may be about 6.0 mg.

The dose of cagrilintide may be about 2.4 mg and the dose of semaglutide may be about 6.9 mg.

30 The dose of cagrilintide may be about 2.4 mg and the dose of semaglutide may be about 7.2 mg.

The dose of cagrilintide may be about 2.4 mg and the dose of semaglutide may be about 8.0 mg.

The dose of cagrilintide may be about 2.4 mg and the dose of semaglutide may be about 12 mg.

The dose of cagrilintide may be about 3.4 mg and the dose of semaglutide may be about 13.5 mg.

Cagrilintide and semaglutide may be administered once-weekly at an initial dose of 0.25 mg and then escalated to the subsequent dosing levels of 0.5 mg, 1.0 mg and 1.7 mg
5 until reaching the target/maintenance dose of 2.4 mg once-weekly.

0.25 mg cagrilintide and semaglutide may be administered once-weekly and escalated every four weeks to the subsequent dosing levels of 0.5 mg, 1.0 mg and 1.7 mg, until reaching the target/maintenance dose of 2.4 mg once-weekly.

0.25 mg cagrilintide and semaglutide may be administered once-weekly and
10 escalated every four weeks to the subsequent dosing levels of 0.5 mg, 1.0 mg and 1.7 mg, until reaching the target/maintenance dose of 2.4 mg once-weekly.

0.25 mg cagrilintide and 0.25 mg semaglutide may be administered once a week for four weeks (weeks 0-3) and escalated every four weeks to the subsequent dosing levels of 0.5 mg cagrilintide and 0.5 semaglutide (weeks 4-7), 1.0 mg cagrilintide and 1.0 semaglutide
15 (weeks 8-11) and 1.7 mg cagrilintide and 1.7 mg semaglutide (weeks 12-15), until reaching the target/maintenance dose of 2.4 mg cagrilintide and 2.4 mg semaglutide mg once-weekly (weeks 16 and thereafter).

Cagrilintide and semaglutide may be administered once-weekly at initial doses of 0.25 mg and then escalated to the subsequent dosing levels of 0.5 mg, 1.0 mg, 1.7 mg and
20 2.4 mg, until reaching the target/maintenance dose of 4.5 mg once-weekly.

Cagrilintide and semaglutide may be administered once-weekly at initial doses of 0.25 mg and then escalated to the subsequent dosing levels of 0.5 mg, 1.0 mg, 1.7 mg, 2.4 mg, 3.6 mg and 4.5 mg, until reaching the target/maintenance dose of 7.2 mg once-weekly.

Cagrilintide and semaglutide may be administered once-weekly at initial doses of
25 0.25 mg and then escalated to the subsequent dosing levels of 0.5 mg, 1.0 mg, 1.7 mg, 2.4 mg, 3.6 mg, 4.5 mg and 7.2 mg, until reaching the target/maintenance dose of 8.0 mg once-weekly.

Cagrilintide and semaglutide may be administered once-weekly at initial doses of 0.25 mg and then escalated to the subsequent dosing levels of 0.5 mg, 1.0 mg, 1.7 mg, 2.4
30 mg, 3.6 mg, 4.5 mg, 7.2 mg and 8.0, until reaching the target/maintenance dose of 16.0 mg once-weekly.

Herein, specific values given in relation to numbers or intervals may be construed as being the specific value or as being the approximate value (such as plus or minus 10, 15 or 20 percent of the specific value, when amounts can be provided by weight; such as plus or
35 minus 0.4, when pH is measured).

Kits

The pharmaceutical formulation disclosed herein may be presented in the form of a kit comprising the pharmaceutical formulation together with instructions for use. The instructions for use may comprise the package insert of a drug.

- 5 The pharmaceutical formulation disclosed herein may be a liquid formulation presented in an injection device. The injection device may be selected from the group consisting of a durable pen, a prefilled pen, or a prefilled syringe.

The pharmaceutical formulation disclosed herein may be a liquid formulation presented in a vial or a cartridge.

- 10 The kit may comprise a dried formulation in a first vial, an aqueous solution in a second vial and instructions for use, wherein reconstitution of the dried formulation in the aqueous solution provides the liquid pharmaceutical formulation disclosed herein.

Following is a non-limiting list of embodiments of the present invention.

15 **EMBODIMENTS**

1. A pharmaceutical formulation comprising an amylin receptor agonist, a GLP-1 receptor agonist, a cyclodextrin comprising hydroxypropyl substitutions and at least one preservative and/or stabilising agent.
- 20 2. The pharmaceutical formulation according to embodiment 1, wherein the GLP-1 receptor agonist has an isoelectric point that is incompatible with the optimal pH of the amylin receptor agonist.
- 25 3. The pharmaceutical formulation according to any one of the preceding embodiments, wherein the optimal pH of the GLP-1 receptor agonist and the optimal pH of the amylin receptor agonist differ by at least about two pH units, such as 2-5 pH units, such as 2-4 pH units, such as 3-5 pH units.
- 30 4. The pharmaceutical formulation according to any of the preceding embodiments, wherein the optimal pH of the amylin receptor agonist is 3.5-4.5, such as about 4.0.
5. The pharmaceutical formulation according to any one of the preceding embodiments, wherein said amylin receptor agonist is cagrilintide.

6. The pharmaceutical formulation according to any of the preceding embodiments, wherein said GLP-1 receptor agonist has an isoelectric point which is equal to or less than 5.0, such as equal to or less than 4.5; such as within the range of 3.0-5.0, such as within the range of 3.5-5.0, such as within the range of 3.5-4.5, such as within the range of 3.8-4.9, such as within the range of 4.0-4.5.

7. The pharmaceutical formulation according to any of the preceding embodiments, wherein said GLP-1 receptor agonist comprises a GLP-1(7-37)-derived peptide having an isoelectric point which is equal to or less than 5.0, such as less than 4.5, such as within the range of 3.0-5.0, such as 3.5-5.0, such as 3.5-4.5, such as 3.8-4.9, such as 4.0-4.5.

8. The pharmaceutical formulation according to embodiment 1, wherein said amylin receptor agonist has a theoretically calculated isoelectric point (pI) within the range of 7.6-9.4 or 8-9.

9. The pharmaceutical formulation according to embodiment 1, wherein said amylin receptor agonist comprises a human amylin-derived peptide which has a theoretically calculated isoelectric point (pI) within the range of 7.6-9.4 or 8-9.

10. The pharmaceutical formulation according to any one of the preceding embodiments, wherein said GLP-1 receptor agonist is semaglutide or tirzepatide.

11. The pharmaceutical formulation according to any one of the preceding embodiments, wherein said GLP-1 receptor agonist is semaglutide.

12. The pharmaceutical formulation according to any one of the preceding embodiments, wherein said cyclodextrin is of the hydroxypropyl-substituted alpha type comprising six ring-arranged glucose units and/or the hydroxypropyl-substituted beta type comprising seven ring-arranged glucose units.

13. The pharmaceutical formulation according to any one of the preceding embodiments, wherein said cyclodextrin is of the hydroxypropyl-substituted alpha type comprising six ring-arranged glucose units.

14. The pharmaceutical formulation according to any one of the preceding embodiments, wherein said cyclodextrin is of the hydroxypropyl-substituted beta type comprising seven ring-arranged glucose units.
- 5 15. The pharmaceutical formulation according to any one of the preceding embodiments, wherein said cyclodextrin comprises a maximum of about 1.2 hydroxypropyls per glucose unit.
- 10 16. The pharmaceutical formulation according to any one of the preceding embodiments, wherein said cyclodextrin comprises a maximum of about 0.92 hydroxypropyls per glucose unit.
- 15 17. The pharmaceutical formulation according to any one of the preceding embodiments, wherein said cyclodextrin comprises a maximum of about 0.75 hydroxypropyls per glucose unit.
18. The pharmaceutical formulation according to any one of the preceding embodiments, wherein said cyclodextrin comprises a maximum of about 0.68 hydroxypropyls per glucose unit.
- 20 19. The pharmaceutical formulation according to any one of the preceding embodiments, wherein said cyclodextrin comprises a minimum of about 0.4 hydroxypropyls per glucose unit.
- 25 20. The pharmaceutical formulation according to embodiment 19, wherein said cyclodextrin comprises a minimum of about 0.58 hydroxypropyls per glucose unit.
21. The pharmaceutical formulation according to embodiment 20, wherein said cyclodextrin comprises about 0.58-1.0 hydroxypropyls per glucose unit.
- 30 22. The pharmaceutical formulation according to embodiment 21, wherein said cyclodextrin comprises an average (MS) of 0.62-0.92 hydroxypropyls per glucose unit.
23. The pharmaceutical formulation according to embodiment 21, wherein said cyclodextrin comprises an average (MS) of about 0.62-0.84 hydroxypropyls per glucose unit.
- 35

24. The pharmaceutical formulation according to embodiment 21, wherein said cyclodextrin comprises an average (MS) of about 0.62 hydroxypropyls per glucose unit.

25. The pharmaceutical formulation according to any one of the preceding
5 embodiments, which is a liquid formulation.

26. The pharmaceutical formulation according to embodiment 25, wherein the pH is about 5.6-6.4, such as about 5.8-6.2, such as about 5.6, such as about 5.7, such as about pH 5.8, such as about 5.9, such as about 6.0, such as about 6.1, such as about 6.2, such as
10 about 6.3, such as about 6.4 of, preferably 5.8-6.2.

27. The pharmaceutical formulation according to any one of embodiments 25-26, comprising more than 10% w/v cyclodextrin.

15 28. The pharmaceutical formulation according to any one of embodiments 25-27, comprising less than 22% w/v cyclodextrin.

29. The pharmaceutical formulation according to any one of embodiments 25-28, comprising less than 20% w/v cyclodextrin.
20

30. The pharmaceutical formulation according to any one of embodiments 25-28, comprising about 10-20% w/v of said cyclodextrin.

31. The pharmaceutical formulation according to any one of embodiments 25-30,
25 comprising about 10-17.5% w/v cyclodextrin.

32. The pharmaceutical formulation according to any one of embodiments 25-31, comprising about 12-18% w/v cyclodextrin.

30 33. The pharmaceutical formulation according to any one of embodiments 25-32, comprising about 11.25-15% w/v cyclodextrin.

34. The pharmaceutical formulation according to any one of embodiments 25-32, comprising about 15% w/v cyclodextrin.
35

35. The pharmaceutical formulation according to any one of embodiments 25-34, comprising at least about 1 mg/ml of said GLP-1 receptor agonist.
36. The pharmaceutical formulation according to any one of embodiments 25-35,
5 comprising a maximum of about 22 mg/ml of said GLP-1 receptor agonist.
37. The pharmaceutical formulation according to any one of embodiments 25-36, comprising about 1-12 mg/ml GLP-1 receptor agonist.
- 10 38. The pharmaceutical formulation according to any one of embodiments 25-37, comprising at least about 1 mg/ml of said amylin receptor agonist.
39. The pharmaceutical formulation according to any one of embodiments 25-38, comprising a maximum of about 30 mg/ml amylin receptor agonist.
15
40. The pharmaceutical formulation according to any one of embodiments 25-39, comprising a maximum of about 22 mg/ml amylin receptor agonist.
41. The pharmaceutical formulation according to any one of embodiments 25-40,
20 comprising about 1-12 mg/ml amylin receptor agonist.
42. The pharmaceutical formulation according to any one of embodiments 25-41, comprising 0.25-22 mg/ml cagrilintide.
- 25 43. The pharmaceutical formulation according to any one of embodiments 25-42, comprising 0.25-22 mg/ml semaglutide.
44. The pharmaceutical formulation according to any one of embodiments 25-42, comprising 5-30 mg/ml tirzepatide.
30
45. The pharmaceutical formulation according to any one of embodiments 25-43, comprising 0.25-22 mg/ml cagrilintide and 0.25-22 mg/ml semaglutide.
46. The pharmaceutical formulation according to any one of embodiments 25-41,
35 comprising an effective amount of cagrilintide and semaglutide or tirzepatide.

47. The pharmaceutical formulation according to any one of the preceding
embodiments, wherein said at least one preservative is phenol and/or m-cresol and/or EDTA.
48. The pharmaceutical formulation according to any one of embodiments 25-47,
5 comprising m-cresol in a concentration of 9-40 mM.
49. The pharmaceutical formulation according to any one of embodiments 25-48,
comprising phenol in a concentration of 18-65 mM.
- 10 50. The pharmaceutical formulation according to any one of embodiments 25-49,
comprising m-cresol in a concentration of 9-40 mM and phenol in a concentration of 18-65
mM.
51. The pharmaceutical formulation according to any one of the preceding
15 embodiments, wherein said at least one stabilising agent is EDTA.
52. The pharmaceutical formulation according to any one of embodiments 25-51,
comprising EDTA in a concentration of 0.5-5.0 mg/ml.
- 20 53. A pharmaceutical formulation comprising cagrilintide, semaglutide, a cyclodextrin of
the hydroxypropyl-substituted alpha and/or beta type, m-cresol, phenol and EDTA; and
having a pH of 5.6-6.4, preferably 5.8-6.2.
54. A pharmaceutical formulation comprising cagrilintide, semaglutide, a cyclodextrin of
25 the hydroxypropyl-substituted alpha and/or beta type and EDTA; and having a pH of 5.6-6.4,
preferably 5.8-6.2.
55. The pharmaceutical formulation according to any one of the preceding
embodiments, further comprising a tonicity agent; with the proviso that the tonicity agent is
30 not sodium chloride.
56. The pharmaceutical formulation according to the previous embodiment, wherein
said tonicity agent is glycerol, mannitol, propylene glycol, sorbitol or trehalose, or a
combination thereof.

57. The pharmaceutical formulation according to embodiment 56, wherein said tonicity agent is glycerol.
58. The pharmaceutical formulation according to any one of embodiment 25-57,
5 comprising glycerol in a concentration of about 2.5-18 mg/ml.
59. The pharmaceutical formulation according to embodiment 56, wherein said tonicity agent is mannitol.
- 10 60. The pharmaceutical formulation according to any one of embodiments 25-59, comprising mannitol in a concentration of about 16.5-37.5 mg/ml, such as about 20 mg/ml.
61. The pharmaceutical formulation according to embodiment 56, wherein said tonicity agent is propylene glycol.
15
62. The pharmaceutical formulation according to any one of embodiments 25-61, comprising propylene glycol in a concentration of about 2-15 mg/ml.
63. The pharmaceutical formulation according to embodiment 56, wherein said tonicity agent is sorbitol.
20
64. The pharmaceutical formulation according to any one of embodiments 25-63, comprising sorbitol in a concentration of about 5-35 mg/ml, such as about 10-30 mg/ml, such as about 16-28 mg/ml, such as about 16.5-25 mg/ml, such as about 16-24 mg/ml, such as
25 about 24 mg/ml, such as about 20 mg/ml, such as about 16 mg/ml, such as about 12 mg/ml.
65. The pharmaceutical formulation according to embodiment 56, wherein said tonicity agent is trehalose.
- 30 66. The pharmaceutical formulation according to any one of embodiments 25-65, comprising trehalose in a concentration of about 33-75 mg/ml, such as about 33-45 mg/ml, such as about 38 mg/ml.
67. The pharmaceutical formulation according to any one of the preceding
35 embodiments, further comprising a buffer having at least one pKa of about 5.0-7.0.

68. The pharmaceutical formulation according to any one of the preceding
embodiments, further comprising a buffer selected from the group consisting of histidine,
citrate and/or phosphate.
- 5
69. The pharmaceutical formulation according to any one of embodiments 25-68,
comprising a maximum of 30 mM buffer.
70. The pharmaceutical formulation according to any one of embodiments 25-69,
10 comprising about 3-30 mM citrate.
71. The pharmaceutical formulation according to any one of embodiments 25-70,
comprising about 3-30 mM histidine, such as 3-15 mM histidine, such as 3-10 mM histidine,
such as about 6 mM histidine.
- 15
72. The pharmaceutical formulation according to any one of embodiments 25-71,
comprising about 3-30 mM phosphate.
73. The pharmaceutical formulation according to any one of the preceding
20 embodiments, further comprising a surfactant.
74. The pharmaceutical formulation according to the preceding embodiment, wherein
said surfactant is polysorbate 20 and/or polysorbate 80.
- 25
75. The pharmaceutical formulation according to any one of embodiments 25-74,
comprising a maximum of about 2.0 mg/ml polysorbate 20 and/or polysorbate 80.
76. The pharmaceutical formulation according to embodiment 75, comprising 0.01-0.1
mg/ml, such as about 0.05 mg/ml polysorbate 20 and/or polysorbate 80; or more than 0.1
30 and less than 0.2 mg/ml polysorbate 20 and/or polysorbate 80.
77. The pharmaceutical formulation according to any one of embodiments 73-76,
wherein said surfactant is polysorbate 80.

78. The pharmaceutical formulation according to the preceding embodiment, comprising 0.01-0.1 mg/ml polysorbate 80; preferably, 0.05 mg/ml polysorbate 80.
79. The pharmaceutical formulation according to any one of embodiments 25-78,
5 comprising at least 75% w/w water, such as about 80% w/w water, such as about 85% w/w water, such as up to about 90% w/w water.
80. The pharmaceutical formulation according to any one of the preceding
10 embodiments, essentially consisting of: an effective amount of cagrilintide and semaglutide;
a cyclodextrin of the hydroxypropyl-substituted alpha and/or beta type, said cyclodextrin comprising 0.58-1.0 hydroxypropyls per glucose unit, phenol and/or m-cresol, EDTA, histidine, sorbitol, polysorbate 20 and/or 80 and about 75-90% w/w water; and having a pH of 5.6-6.4, preferably about 5.8-6.2.
- 15 81. The pharmaceutical formulation according to any one of the preceding
embodiments, essentially consisting of: an effective amount of cagrilintide and semaglutide;
a cyclodextrin of the hydroxypropyl-substituted alpha and/or beta type, said cyclodextrin comprising an average of 0.62-0.92 hydroxypropyls per glucose unit; phenol and/or m-cresol, EDTA, histidine, sorbitol, polysorbate 20 and/or 80 and about 75-90% w/w water; and having
20 a pH of 5.6-6.4, preferably about 5.8-6.2.
82. A pharmaceutical formulation comprising:
– 0.25-22 mg/ml cagrilintide,
– 0.25-22 mg/ml semaglutide,
25 – more than 10% w/v and less than 22% w/v, such as 10-20% w/v cyclodextrin of the hydroxypropyl-substituted alpha and/or beta type, wherein said cyclodextrin comprises 0.58-1.0 hydroxypropyls per glucose unit,
– about 18-65 mM phenol and/or 9-40 mM m-cresol and/or 0.5-5.0 mg/ml EDTA,
– a buffer having at least one pKa value of about 5.0-7.0,
30 – about 5-35 mg/ml sorbitol,
– up to 1.0 mg/ml polysorbate 20 and/or 80, such as 0.01-0.1 mg/ml, such as about 0.05 mg/ml polysorbate 20 and/or 80, or more than 0.1 and less than 0.2 mg/ml polysorbate 20 and/or 80,
– about 75-90% w/w water
35 and having a pH of 5.6-6.4, such as 5.7-6.4, preferably 5.8-6.2.

83. A pharmaceutical formulation comprising:
- 0.25-22 mg/ml cagrilintide,
 - 0.25-22 mg/ml semaglutide,
 - 5 - more than 10% w/v and less than 22% w/v, such as 10-20% w/v cyclodextrin of the hydroxypropyl-substituted alpha and/or beta type, wherein said cyclodextrin comprises 0.58-1.0 hydroxypropyls per glucose unit,
 - about 18-65 mM phenol and/or 9-40 mM m-cresol and/or 0.5-5.0 mg/ml EDTA,
 - about 3-30 mM histidine,
 - 10 - about 5-35 mg/ml sorbitol,
 - up to 1.0 mg/ml polysorbate 20 and/or 80, such as 0.01-0.1 mg/ml, such as about 0.05 mg/ml polysorbate 20 and/or 80, or more than 0.1 and less than 0.2 mg/ml polysorbate 20 and/or 80,
 - about 75-90% w/w water
 - 15 and having a pH of 5.6-6.4, such as about 5.7-6.4, preferably about 5.8-6.2.
84. A pharmaceutical formulation comprising:
- 0.25-22 mg/ml cagrilintide,
 - 0.25-22 mg/ml semaglutide,
 - 20 - more than 10% w/v and less than 22% w/v, such as 10-20% w/v cyclodextrin of the hydroxypropyl-substituted alpha and/or beta type, wherein said cyclodextrin comprises 0.58-1.0 hydroxypropyls per glucose unit,
 - about 18-65 mM phenol and/or 9-40 mM m-cresol and/or 0.5-5.0 mg/ml EDTA,
 - about 3-30 mM citrate,
 - 25 - about 5-35 mg/ml sorbitol,
 - up to 1.0 mg/ml polysorbate 20 and/or 80, such as 0.01-0.1 mg/ml, such as 0.05 mg/ml polysorbate 20 and/or 80, or more than 0.1 and less than 0.2 mg/ml polysorbate 20 and/or 80,
 - about 75-90% w/w water,
 - 30 and having a pH of 5.6-6.4, such as about 5.7-6.4, preferably about 5.8-6.2.
85. The pharmaceutical formulation according to any one of the preceding embodiments, which essentially consists of:
- an effective amount of cagrilintide and semaglutide,

- more than 10% w/v and less than 22% w/v, such as 10-20% w/v cyclodextrin of the hydroxypropyl-substituted alpha and/or beta type, wherein said cyclodextrin comprises 0.58-1.0 hydroxypropyls per glucose unit,
- about 3-30 mM histidine,
- 5 – about 5-35 mg/ml sorbitol,
- up to 1.0 mg/ml polysorbate 20 and/or 80, such as 0.01-0.1 mg/ml, such as 0.05 mg/ml polysorbate 20 and/or 80, or more than 0.1 and less than 0.2 mg/ml polysorbate 20 and/or 80,
- a pH of 5.6-6.4, such as about 5.7-6.4, preferably about 5.8-6.2,
- 10 – water for injection.

86. The pharmaceutical formulation according to any one of the preceding embodiments, which essentially consists of:

- 0.25-22 mg/ml cagrilintide,
- 15 – 0.25-22 mg/ml semaglutide,
- more than 10% w/v and less than 22% w/v, such as 10-20% w/v cyclodextrin of the hydroxypropyl-substituted alpha and/or beta type, wherein said cyclodextrin comprises 0.58-1.0 hydroxypropyls per glucose unit,
- about 3-30 mM, preferably about 6 mM histidine,
- 20 – about 5-35 mg/ml sorbitol,
- up to 1.0 mg/ml polysorbate 20 and/or 80; such as 0.01-0.1 mg/ml, such as 0.05 mg/ml; or more than 0.1 and less than 0.2 mg/ml or about 0.1-0.2 mg/ml polysorbate 20 and/or 80,
- a pH of 5.6-6.4, such as about 5.7-6.4, preferably about 5.8-6.2,
- 25 – water for injection.

87. The pharmaceutical formulation according to any one of the preceding embodiments for use as a medicament.

- 30 88. The pharmaceutical formulation according to any one of embodiments 1-86 for use in the treatment or prevention of obesity.

89. The pharmaceutical formulation according to any one of embodiments 1-86 for use in the treatment or prevention of overweight in a subject with at least one weight-related co-morbidity.
- 35

90. The pharmaceutical formulation according to any one of embodiments 1-86 for use in the treatment or prevention of at least one weight-related co-morbidity in a subject that is overweight or obese at the start of treatment.

5 91. The pharmaceutical formulation according to any one of embodiments 1-86 for use in the treatment or prevention of obesity in a subject, said subject having an initial body mass index (BMI) of 25 kg/m² or more, 27 kg/m² or more, 28 kg/m² or more, or 30 kg/m² or more.

10 92. The pharmaceutical formulation according to any one of embodiments 1-86, for use in the treatment of overweight in a subject, said subject having an initial body mass index (BMI) of 24 kg/m² or more, 25 kg/m² or more or 27 kg/m² or more and at least one weight-related co-morbidity.

15 93. The pharmaceutical formulation according to any one of embodiments 1-86, for use, as an adjunct to a reduced-calorie diet and increased physical activity, in the treatment or prevention of overweight or obesity in an adult subject with: an initial body mass index (BMI) of 25 kg/m², 27 kg/m², 28 kg/m² or 30 kg/m² or more (obesity); or an initial body mass index (BMI) of 24 kg/m², 25 kg/m² or 27 kg/m² or more (overweight) and at least one weight-related co-morbidity.

20 94. The pharmaceutical formulation according to any one of embodiments 1-86, for use, as an adjunct to a reduced-calorie diet and increased physical activity, in the chronic weight management of adult subjects with an initial body mass index (BMI) of 30 kg/m² or greater (obesity) or 27 kg/m² or greater (overweight) in the presence of at least one weight-related co-morbidity.

30 95. Use of the liquid pharmaceutical formulation according to any one of embodiments 87-94, wherein said at least one co-morbidity is diabetes and/or one or more cardiovascular diseases (CVD) and/or non-alcoholic steatohepatitis (NASH) or metabolic dysfunction-associated steatohepatitis (MASH) and/or alcoholic liver disease (ALD) and/or and/or obstructive sleep apnoea.

35 96. The pharmaceutical formulation according to any one of embodiments 1-86, for use in the treatment of a subject with diabetes, such as type II diabetes.

97. The pharmaceutical formulation according to any one of embodiments 1-86, for use in the treatment of a subject with diabetes, such as type II diabetes, and at least one diabetes-related co-morbidity.
- 5 98. The pharmaceutical formulation according to any one of embodiments 1-86, for use as an adjunct to diet and exercise to improve glycemic control in adults with type 2 diabetes mellitus.
99. The pharmaceutical formulation according to any one of embodiments 1-86, for use
10 in the treatment and/or prevention of chronic kidney disease (CKD) in a subject with diabetes.
100. The pharmaceutical formulation according to any one of embodiments 1-86, for use
15 in the treatment and/or prevention of one or more cardiovascular diseases (CVD).
101. The pharmaceutical formulation according to any one of embodiments 1-86, for use in the treatment and/or prevention of non-alcoholic steatohepatitis (NASH), metabolic dysfunction-associated steatohepatitis (MASH) and/or alcoholic liver disease (ALD).
- 20 102. The pharmaceutical formulation according to any one of embodiments 1-86, for use in the treatment and/or prevention of obstructive sleep apnoea in a subject that is overweight or obese at the start of treatment.
103. The pharmaceutical formulation according to any one of embodiments 1-86, for use
25 in the treatment and/or prevention of cognitive impairment, such as that caused by Alzheimer's disease.
104. The pharmaceutical formulation according to any one of embodiments 1-86 for use according to any one of embodiments 87-103, characterised in that the formulation is administered by parenteral injection.
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105. The pharmaceutical formulation according to any one of embodiments 1-86 for use according to any one of embodiments 87-103, characterised in that the formulation is administered by subcutaneous injection.

106. The pharmaceutical formulation according to any one of embodiments 1-86 for use according to any one of embodiments 87-103, characterised in that the formulation is administered approximately once a week.
- 5 107. The pharmaceutical formulation according to any one of embodiments 1-86 for use according to any one of embodiments 87-106, wherein the ratio of the dose of cagrilintide administered to the dose of semaglutide administered is about 1:1.
108. The pharmaceutical formulation according to any one of embodiments 1-86 for use
10 according to any one of embodiments 87-106, wherein the ratio of the dose of cagrilintide administered to the dose of semaglutide administered is from 1:1 to 1:7.
109. The pharmaceutical formulation according to any one of embodiments 1-86 for use according to any one of embodiments 87-106, wherein the ratio of the dose of cagrilintide
15 administered to the dose of semaglutide administered is about 1:2.
110. A kit comprising the pharmaceutical composition as defined in any one of embodiments 1-86 and instructions for use.
- 20 111. A kit comprising a vial comprising the pharmaceutical formulation according to any one of embodiments 1-86, and instructions for use.
112. The kit according to embodiment 110, wherein said pharmaceutical formulation is a liquid pharmaceutical formulation.
- 25 113. The kit according to any one of embodiments 110-112, said liquid pharmaceutical formulation comprising:
- 0.25-22 mg/ml cagrilintide,
 - 0.25-22 mg/ml semaglutide,
 - 30 - more than 10% w/v and less than 22% w/v, such as 10-20% w/v, cyclodextrin of the hydroxypropyl-substituted alpha and/or beta type, wherein said cyclodextrin comprises 0.58-1.0 hydroxypropyls per glucose unit,
 - about 18-65 mM phenol and/or 9-40 mM m-cresol and/or 0.5-5.0 mg/ml EDTA,
 - a buffer having at least one pKa value of about 5.0-7.0, such as 3-30 mM
35 histidine or citrate

- about 5-35 mg/ml sorbitol,
 - more than 0.1 and less than 0.2 mg/ml polysorbate 20 and/or 80,
 - about 75-90% w/w water and
- having a pH of 5.6-6.4, such as about 5.7-6.4, preferably about 5.8-6.2.

5

114. The kit according to embodiment 110, comprising:

- a freeze-dried or spray-dried formulation in a first vial,
- an aqueous solution in a second vial and
- instructions for use,

10

wherein mixture of the contents of the two vials provides the liquid pharmaceutical formulation according to any one of embodiments 25-86.

115. A kit comprising a freeze-dried or spray-dried formulation in a first vial, an aqueous solution in a second vial and instructions for use,

15

wherein the formulation in the first vial comprises:

- cagrilintide,
- semaglutide,
- cyclodextrin of the hydroxypropyl-substituted alpha and/or beta type, wherein said cyclodextrin comprises 0.58-1.0 hydroxypropyls per glucose unit,
- optionally, EDTA,
- optionally, a buffer having at least one pKa value of about 5.0-7.0,
- optionally, sorbitol,
- polysorbate 20 and/or 80;

20

and wherein the aqueous solution in the second vial comprises

25

- water for injection,
- optionally, phenol and/or m-cresol,
- optionally, EDTA,
- optionally, a buffer having at least one pKa value of about 5.0-7.0, such as histidine or citrate,

30

- optionally, sorbitol.

116. The kit according to embodiment 115, wherein dissolution of the freeze-dried or spray-dried formulation in the aqueous solution provides a liquid pharmaceutical formulation comprising:

35

- 0.25-22 mg/ml cagrilintide,

- 5
- 10
- 0.25-22 mg/ml semaglutide,
 - more than 10% w/v and less than 22% w/v, such as 10-20% w/v cyclodextrin of the hydroxypropyl-substituted alpha and/or beta type, wherein said cyclodextrin comprises 0.58-1.0 hydroxypropyls per glucose unit,
 - about 18-65 mM phenol and/or 9-40 mM m-cresol and/or 0.5-5.0 mg/ml EDTA,
 - a buffer having at least one pKa value of about 5.0-7.0, such as histidine or citrate,
 - about 5-35 mg/ml sorbitol,
 - more than 0.1 and less than 0.2 mg/ml polysorbate 20 and/or 80,
 - about 75-90% w/w water andhaving a pH of 5.6-6.4, such as about 5.7-6.4, preferably about 5.8-6.2.

117.

15

A kit comprising the pharmaceutical composition as defined in any one of embodiments 1-86 and an injection device for administration of said pharmaceutical formulation to a subject, wherein said injection device is selected from the group consisting of a durable pen, a prefilled pen and a prefilled syringe.

EXAMPLES

20

EXAMPLE 1: EFFECT OF HYDROXYPROPYL-BETA-CYCLODEXTRIN (HP-B-CD) ON THE CHEMICAL STABILITY OF CAGRILINTIDE

This example demonstrated the ability of HP-B-CD to chemically stabilise cagrilintide, chemical stability being measured in terms of cagrilintide purity and cagrilintide-related high molecular weight protein (HMWP).

Cagrilintide is optimally stable at pH 4.0, the rate of its chemical degradation typically accelerating with an increase in pH. Surprisingly, a stable cagrilintide formulation was obtained at pH 6 when it was formulated with HP-B-CD.

Composition

The compositions of cagrilintide formulations 1, 2 and 3 are shown in table 1.

30

Table 1 Composition of cagrilintide formulations 1, 2 and 3

Ingredient	Cagrilintide formulation		
	1	2	3

Cagrilintide drug substance (mg/ml)	18	18	18
HP-B-CD			
KLEPTOSE® (Roquette) (% w/v) (Average MS: 0.92, MS range: 0.81-0.99) ¹	0	0	25
Citrate, 1 H ₂ O ² (mM)	6.1	3.7	3.7
Disodium hydrogen phosphate, 2 H ₂ O ² (mM)	7.7	12.6	12.6
HCl	q.s.	q.s.	q.s.
NaOH	q.s.	q.s.	q.s.
Water for injection (WFI)	To make 1 ml	To make 1 ml	To make 1 ml
pH	4.0	6.0	6.0

¹ MS: molar substitution, corresponds to hydroxypropyls per glucose unit
² Different buffer concentrations across formulations are used to ensure buffering at different pH

5 **Preparation process**

Each cagrilintide formulation was prepared by first dissolving the excipients in water and then dissolving cagrilintide drug substance in the excipient solution. The solution was pH adjusted and water was added to reach the final desired volume before being sterilised by filtration through a 0.22 µm sterile filter. After filtration, the formulation was filled in a 1 ml

10 prefilled syringe.

Methods

Samples were stored at 37°C for up to 21 days. After 14 days and 21 days, samples were analysed to determine the HMWP and cagrilintide purity levels.

15 Levels of covalently bound HMWP were quantified using size exclusion chromatography (SEC). Samples were analysed using a WATERS HMWP column (7.8 x 300mm) with an isocratic elution consisting of 500 mM sodium chloride, 10 mM sodium dihydrogen phosphate monohydrate, 5 mM ortho-phosphate and 50% (v/v) isopropanol. Chromatography was conducted with UV detection (215 nm) at 50°C using a 10 µl injection

20 volume and a flow rate of 0.5 ml/min. HMWP was quantified as being the area of all components eluting before the main peak divided by the area of the main peak x 100%.

Cagrilintide purity was determined using reversed phase ultra-high performance liquid chromatography (RP-UHPLC). Samples were analysed using a Kinetex C18, 1.7 µm,

100 Å, column (2.1 x 150 mm) with a gradient elution of eluent A consisting of 90% v/v 0.09 M phosphate solution, pH 3.6 and 10% v/v acetonitrile, and eluent B consisting of 60% v/v acetonitrile and 20% v/v isopropanol. Chromatography was conducted with UV detection (215 nm) at 60°C using 2-7.5 µl injection volume and a flow rate of 0.25 ml/min. Purity was evaluated as the area of the main peak divided by the area of all peaks x 100%.

Table 2 Chemical purity (%) of cagrilintide at pH 4.0 and 6.0 with and without HP-B-CD

Cagrilintide formulation	pH	HP-B-CD (MS: 0.92) ¹	Levels of HMWP (%) as a function of time (days) at 37°C			Purity (%) of cagrilintide as a function of time (days) at 37°C		
			0	14	21	0	14	21
1	4.0	0% w/v	0.0%	0.2%	0.2%	90.4%	88.2%	87.4%
2	6.0	0% w/v	0.1%	7.7%	9.8%	90.1%	74.0%	67.1%
3	6.0	25% w/v	0.1%	0.3%	0.5%	90.2%	79.9%	75.3%

¹MS: molar substitution, corresponds to hydroxypropyls per glucose unit

Concluding Remarks

Table 2 shows that when cagrilintide was stored at 37°C and at a pH of 4.0, very little HMWP was formed and only a minor decrease in cagrilintide purity was seen. In contrast, when the pH was 6.0 the rate of HMWP formation and decrease in cagrilintide purity accelerated. Surprisingly, this rapid chemical degradation was counteracted by the addition of HP-B-CD to the formulation, making it possible to formulate cagrilintide at pH 6.

EXAMPLE 2: EFFECT OF HP-B-CD ON SEMAGLUTIDE PHYSICAL STABILITY

This example demonstrates the ability of HP-B-CD to physically stabilise semaglutide, which has a propensity to form peptide fibrils. The effect was evident when semaglutide was formulated at suboptimal pH.

Composition

The compositions of semaglutide formulations 1, 2 and 3 are shown in table 3.

Table 3 Composition of semaglutide formulations 1, 2 and 3

Ingredient	Semaglutide formulation		
	1	2	3
Semaglutide drug substance (mg/ml)	4.8	4.8	4.8
HP-B-CD KLEPTOSE® (Roquette) (% w/v) (Average MS: 0.92, MS range: 0.81-0.99) ¹	0	25	0
Citrate, 1 H ₂ O ² (mM)	3.7	3.7	0.9
Disodium hydrogen phosphate, 2 H ₂ O ² (mM)	12.6	12.6	18.2
HCl	q.s.	q.s.	q.s.
NaOH	q.s.	q.s.	q.s.
WFI	To make 1 ml	To make 1 ml	To make 1 ml
pH	6.0	6.0	7.4

¹ MS: molar substitution, corresponds to hydroxypropyls per glucose unit
² Different buffer concentrations across formulations are used to ensure buffering at different pH

5

Preparation process

Formulations were prepared as described in example 1.

Methods

10 The propensity of semaglutide to aggregate and form peptide fibrils, parameters used to quantify physical stability, was measured using a Thioflavin T (ThT) fluorescence stress assay. The analysis for presence of peptide fibrils is based on the fluorescence characteristics of the ThT probe, which displays low fluorescence in the unbound state/native peptide-bound state but high fluorescence when bound to peptide fibrils as well as a red shift
15 in the wavelength of maximum fluorescence upon fibril binding.

 Two samples were pooled and 1400 µl sample was added to 28 µl 1mM ThT stock solution, of which 200 µl was then transferred to 6 different wells on a 96 well microtiter plate with a glass bead in. The assay was run with double orbital shaking and a speed of 300 rpm at 40°C for 169 hours on a BMG CLARIOstar fluorescence plate reader equipped with
20 monochromators for both excitation and emission using 450 nm and 480 nm, respectively. The lag time was measured from the start of the experiment until fibrillation occurs, shown as an increase in ThT fluorescence.

Table 4 Physical stability for semaglutide at pH 6.0 and 7.4

Semaglutide formulation	pH	HP-B-CD (MS: 0.92) ³	Lag time until fibrillation
1	6.0	0% w/v	2.35 hours ¹
2	6.0	25% w/v	>169 hours ^{1,2}
3	7.4	0% w/v	>169 hours ^{1,2}

¹ Result is the mean of 6 replicates

² Fibrillation was not observed in any of the 6 replicates within the 169 hours' duration of the experiment

³ MS: molar substitution, corresponds to hydroxypropyls per glucose unit

Concluding Remarks

The semaglutide formulations were subjected to shear stress-inducing conditions and the propensity of semaglutide to form peptide fibrils was measured. Surprisingly, the presence of HP-B-CD was found to inhibit semaglutide peptide fibril formation. When semaglutide was formulated at pH 6 and in the absence of HP-B-CD (semaglutide formulation 1), fibrillation occurred after 2.35 hours; that is, semaglutide was not physically stable. However, when semaglutide was formulated at pH 6 and in the presence of HP-B-CD (semaglutide formulation 2), no fibrillation was observed throughout the duration of the experiment; that is, semaglutide was physically stable. Furthermore, the physical stability of semaglutide, when formulated at pH 6 and in the presence of HP-B-CD (semaglutide formulation 2), was found comparable to the physical stability of semaglutide when formulated in the absence of HP-B-CD but at its optimal formulation conditions in terms of pH 7.4 (semaglutide formulation 3).

EXAMPLE 3: EFFECT OF HP-B-CD ON SEMAGLUTIDE CHEMICAL STABILITY

This example demonstrated the ability of HP-B-CD to chemically stabilise semaglutide, chemical stability being measured in terms of semaglutide purity and semaglutide-related high molecular weight protein (HMWP).

Composition

The same formulations were used as in example 2.

Preparation process

Formulations were prepared as described in example 1.

Methods

Levels of HMWP and semaglutide purity were determined after 0 days, 14 days, and 21 days' storage at 37°C.

Semaglutide purity was determined using reversed phase high performance liquid chromatography (RP-HPLC) where the samples were analysed using a Kinetex C18, 2.6 µm column (4.6 x 150 mm) with a gradient elution of eluent A consisting of 90% v/v 0.09 M phosphate solution, pH 3.6 and 10% v/v acetonitrile, and eluent B consisting of 60% v/v acetonitrile and 20% v/v isopropanol. Chromatography was conducted with UV detection (210 nm) at 30°C using a 10-100 µl injection volume and a flow rate of 0.7 ml/min. Purity was quantified as being the area of the main peak divided by the area of all peaks x 100%.

The level of covalently bound HMWP was determined using size exclusion chromatography (SEC). Samples were analysed using a Waters SEC 1.7 µm column (4.6 x 150 mm) with an isocratic elution consisting of 300 mM sodium chloride, 10 mM sodium dihydrogen phosphate, 5 mM ortho-phosphate and 50% v/v 2-propanol. Chromatography was conducted with UV detection (280 nm) at 50°C using a 1-10 µl injection volume and a flow rate of 0.3 ml/min. HMWP was quantified as being the area of all components eluting before the main peak divided by the area of the main peak x 100%.

Table 5 Semaglutide purity at suboptimal and optimal pH

Semaglutide formulation	pH	HP-B-CD (MS: 0.92) ¹	Purity (%) of semaglutide as a function of time (days) at 37°C			Levels of HMWP (%) as a function of time (days) at 37°C		
			0	14	21	0	14	21
1	6.0	0% w/v	96.89%	94.58%	93.48%	0.12%	0.25%	0.35%
2	6.0	25% w/v	96.51%	94.53%	93.73%	0.04%	0.11%	0.11%
3	7.4	0% w/v	96.82%	95.56%	94.81%	0.12%	0.32%	0.40%

¹ MS: molar substitution, corresponds to hydroxypropyls per glucose unit

Concluding Remarks

The results in table 5 show that the chemical purity of semaglutide decreased over time. The chemical purity of semaglutide decreased more rapidly when it was formulated at pH 6.0 (semaglutide formulation 1) than when it was formulated at its optimal pH 7.4 (semaglutide formulation 3). Surprisingly, HP-B-CD improved the chemical stability of semaglutide (in terms of purity decline and HMWP formation) when it was formulated at pH 6.0 (semaglutide formulation 2).

EXAMPLE 4: EFFECT OF HYDROXYPROPYL-B-CYCLODEXTRIN CONCENTRATION ON SEMAGLUTIDE CHEMICAL STABILITY

This example shows the concentration-dependent effect of HP-B-CD on the chemical stability of semaglutide.

Composition

The composition of co-formulations comprising different concentrations of HP-B-CD is shown in table 6.

Table 6 Composition of co-formulation comprising different concentrations of HP-B-CD

Ingredient	Co-formulation		
	1	2	3
Cagrilintide drug substance (mg/ml)	3.2	3.2	3.2
Semaglutide drug substance (mg/ml)	3.2	3.2	3.2
HP-B-CD KLEPTOSE® (Roquette) (% w/v) (Average MS: 0.62, MS range: 0.58-0.68) ¹	11.25	12.5	15
L-Histidine (mM)	6	6	6
Sorbitol ² (mg/ml)	26	24	20
Polysorbate 80 (mg/ml)	0.05	0.05	0.05
HCl	q.s.	q.s.	q.s.
NaOH	q.s.	q.s.	q.s.
WFI	To make 1 ml	To make 1 ml	To make 1 ml
pH	5.7	5.7	5.7

¹ MS: molar substitution, corresponds to hydroxypropyls per glucose unit
² Different sorbitol concentrations are needed to obtain isotonicity because of the varying HP-B-CD concentrations tested

Preparation

Formulations were prepared as described in example 1.

Method

5 Samples were stored at 37°C for 28 days at which samples were analysed to
determine the chemical purity of semaglutide after 14, 21, and 28 days.

 The purity of semaglutide was determined using reversed phase ultra-high
performance liquid chromatography (RP-UHPLC), where the samples were analysed using a
Waters Acquity phenyl-hexyl 1.7µm column (2.1 x 150mm) with a gradient elution of eluent A
10 consisting of 0.09% TFA in MQ water, and eluent B consisting of 0.09% TFA in 80%
acetonitrile. Chromatography was conducted with UV detection (215nm) at 62°C using 2-14µl
injection volume and a flow rate of 0.25ml/min. Purity was evaluated as the area of the main
peak of semaglutide divided by the area of all related peaks x 100%.

 Note that, in other experiments, the same method was used to determine cagrilintide
15 purity.

Table 7 Chemical purity (%) for semaglutide with different HP-B-CD concentrations

Co-formulation	HP-B-CD (MS: 0.62) ¹	Semaglutide purity (%) as a function of time (days) at 37°C			
		0	14	21	28
1	11.25% w/v	96.90%	90.72%	88.65%	87.52%
2	12.5% w/v	96.87%	92.90%	91.25%	89.81%
3	15% w/v	96.83%	93.61%	92.92%	91.67%

¹ MS: molar substitution, corresponds to hydroxypropyls per glucose unit

Concluding Remarks

20 The results in table 7 show that the chemical stability and thus purity of semaglutide
also depended on HP-B-CD concentration. Semaglutide remained chemically stable in all of
the co-formulations (comprising 11.25-15% w/v HP-B-CD). However, semaglutide chemical
25 stability and thus purity was highest when the co-formulation comprised 15% w/v HP-B-CD.

EXAMPLE 5: EFFECT OF DIFFERENT TONICITY AGENTS ON CO-FORMULATION PHYSICAL STABILITY

This example shows the stabilising effect of different tonicity agents on the physical stability of otherwise identical cagrilintide and semaglutide co-formulations.

Composition

The composition of co-formulations comprising different tonicity agents is shown in table 8.

10 **Table 8** **Composition of co-formulations comprising different tonicity agents**

[illegible]

WFI	To make 1ml	To make 1ml	To make 1ml	To make 1ml	To make 1ml	To make 1ml	To make 1ml
pH	5.7	5.7	5.7	5.7	5.7	5.7	5.7

¹ MS: molar substitution, corresponds to hydroxypropyls per glucose unit

Preparation process

Formulations were prepared as described in example 1.

5

Methods

All samples were stored for at stressed condition defined as:

- Duration: 18 days
- Temperature: 37°C
- Stress condition: During storage, samples were invented 360° to simulate patient use out of refrigerated storage. The rotations were performed 100 times, five days a week.

10

The number of sub-visible particles present quantifies the physical stability of cagrilintide and semaglutide combined and were obtained by means of micro-flow imaging (MFI, see e.g. Sharma, D.K. et al. AAPS J. (2010), 12: 455-464 for principles of the MFI technique). The following procedure was employed for each analysed syringe sample: The experiment was performed at ambient temperature. The liquid from each syringe was taken out by first removing the plunger and then pipetting the liquid into the sample container. The sample was transferred to a 96 deep-well plate which was inserted into the sample handling unit (Bot1) of a Protein Simple MFI™ 5200 apparatus equipped with a standard Protein Simple MFI™ 100 µm flow cell. The sample was analysed by standard MFI system settings implying that the liquid was pipetted into a reservoir connected to a flow cell, the liquid was illuminated by a 10 LED light source (470 nm), and a digital camera (via magnification optics) recorded the contents of the flow cell as bright field images throughout the experiment. Data acquisition was accomplished using Protein Simple MVSS software. The recorded image stream from the entire run was processed by validated Novo Nordisk proprietary software MFI Data Validator whereby the number (normalised to counts per ml analysed liquid) of individual particles was obtained and presented by size; >5 µm, >10 µm, and >25 µm which are standard size ranges for sub-visible particles. Note that the number of particles >5 µm includes all particles greater than 5 µm in diameter (>5 µm, >10 µm and >25 µm) and the

15

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number of particles > 10 μm includes all particles greater than 10 μm in diameter (>10 μm and >25 μm). The particle size is defined as the equivalent circular diameter (ECD).

Table 9 The effect of different tonicity agents on co-formulation physical stability

Co- formulation	Tonicity agent	Number of sub-visible particles as a function of time (days)				
		0	7	11	14	18
Particle size >5µm						
4	None	256	385	429	952	1296
5	Glycerol	292	241	292	466	691
6	Sorbitol	212	341	340	517	345
7	Mannitol	94	438	177	245	360
8	Trehalose	121	155	213	504	472
9	Sucrose	59	104	140	539	749
10	NaCl ¹	36	946	-	-	-
Particle size >10µm						
4	None	4	27	96	216	377
5	Glycerol	10	23	23	48	106
6	Sorbitol	6	54	42	36	73
7	Mannitol	2	23	12	8	50
8	Trehalose	4	10	40	50	69
9	Sucrose	2	8	13	142	253
10	NaCl ¹	2	403	-	-	-
Particle size >25µm						
4	None	0	4	4	27	75
5	Glycerol	0	0	4	6	4
6	Sorbitol	0	0	2	4	0
7	Mannitol	0	0	0	0	0
8	Trehalose	0	0	0	0	4
9	Sucrose	0	0	2	15	55
10	NaCl ¹	0	106	-	-	-

5 Results are the mean of 2 replicates and has been rounded to nearest integer value

(-) Sampling not performed

¹ For co-formulation 10 with NaCl, sampling was discontinued earlier than for the other formulations because of the rapid increase in sub-visible particle counts.

5 **Concluding Remarks**

 The results in table 9 show that the sub-visible particle count increased most rapidly in the co-formulation comprising NaCl as tonicity agent (co-formulation 10). After 7 days, the particle count vastly exceeded the particle count determined for the other co-formulations. Therefore, sampling for the analysis of number of sub-visible particles was discontinued for
10 the NaCl-containing co-formulation after 7 days.

 After 14 days an increase in sub-visible particle count was seen in the co-formulations containing glycerol and sucrose and the two co-formulations were deemed comparable regarding physical stability. The particle count remained lowest in the co-formulations that contained mannitol, sorbitol or trehalose. In these co-formulations, virtually
15 no increase in the number of sub-visible particles was seen during the 18 days that the co-formulations were stored at stressed conditions.

 Of the co-formulations tested, those comprising mannitol, sorbitol or trehalose as tonicity agent remained the most stable over time.

20 **EXAMPLE 6: EFFECT OF DIFFERENT SURFACTANTS ON CO-FORMULATION PHYSICAL STABILITY**

 This example shows the effect of different surfactants on the physical stability of otherwise identical cagrilintide and semaglutide co-formulations.

Composition

25 The compositions of co-formulations comprising different types of surfactants are shown in table 10.

Table 10 Composition of co-formulation comprising different surfactants

Ingredient	Co-formulation		
	11	12	13
Cagrilintide drug substance (mg/ml)	3.2	3.2	3.2
Semaglutide drug substance (mg/ml)	3.2	3.2	3.2
HP-B-CD KLEPTOSE® (Roquette) (% w/v)	10	10	10

(Average MS: 0.62, MS range: 0.58-0.68) ¹			
L-Histidine (mM)	6	6	6
Polysorbate 20 (mg/ml)	0.05	-	-
Polysorbate 80 (mg/ml)	-	0.05	-
Poloxamer 188 (mg/ml)	-	-	0.5
HCl	q.s.	q.s.	q.s.
NaOH	q.s.	q.s.	q.s.
WFI	To make 1 ml	To make 1 ml	To make 1 ml
pH	5.7	5.7	5.7

¹ MS: molar substitution, corresponds to hydroxypropyls per glucose unit

Preparation process

Formulations were prepared as described in example 1.

5

Methods

All samples were stored for at stressed condition defined as:

- Duration: 17 days
- Temperature: 37°C ± 2°C
- Stress condition: During storage, samples were invented 360° to simulate patient use out of refrigerated storage. The rotations were performed 100 times, five days every week.

10

The number of sub-visible particles was quantified as described in example 5.

15 Table 11 The effect of different surfactants on co-formulation physical stability

Co- formulation	Surfactant	Number of sub-visible particles as a function of time point (days)				
		0	7	10	14	17
Particle size >5µm						
11	Polysorbate 20	464	304	396	772	2803
12	Polysorbate 80	566	292	520	286 ¹	348

13	Poloxamer 188	1235	1278	2783	940 ¹	1704
Particle size >10µm						
11	Polysorbate 20	23	23	43	143	297
12	Polysorbate 80	19	31	76	57 ¹	48
13	Poloxamer 188	100	245	301	290 ¹	523
Particle size >25µm						
11	Polysorbate 20	0	2	6	11	13
12	Polysorbate 80	0	0	0	4 ¹	0
13	Poloxamer 188	0	13	17	61 ¹	52

Results are the mean of 2 replicates and has been rounded to nearest integer value

¹ Only one replicate was performed

Concluding Remarks

5 Co-formulation 12 contained the lowest number of sub-visible particles when stored for 17 days under stressed conditions. In co-formulation 11, containing polysorbate 20, an increase in sub-visible particles was observed after 14 days, while in co-formulation 13 containing poloxamer 188 sub-visible particles are formed after 7 days at stressed conditions. It is evident that the co-formulation containing polysorbate 80 was the most stable
10 and that the co-formulation containing polysorbate 20 was also acceptably stable.

EXAMPLE 7: EFFECT OF DIFFERENT BUFFER SUBSTANCES ON CO-FORMULATION PHYSICAL STABILITY

 This example shows that the buffer substance has an effect on the physical stability of an otherwise identical cagrilintide and semaglutide co-formulation.

15

Composition

The composition of co-formulation 1 and co-formulation 14 are shown in table 12.

Table 12 Composition of co-formulation 1 and co-formulation 14

Ingredient	Co-formulation	
	1	14
Cagrilintide drug substance (mg/ml)	3.2	3.2
Semaglutide drug substance (mg/ml)	3.2	3.2
HP-B-CD KLEPTOSE® (Roquette) (% w/v) (Average MS: 0.92, MS range: 0.81-0.99) ¹	25	25
Citrate, 1 H ₂ O (mM)	3	-
L-Histidine (mM)	-	6
HCl	q.s.	q.s.
NaOH	q.s.	q.s.
WFI	To make 1 ml	To make 1 ml
pH	5.7	5.7

¹ MS: molar substitution, corresponds to hydroxypropyls per glucose unit

Preparation process

- 5 Formulations were prepared as described in example 1.

Methods

All samples were stored for at stressed condition defined as:

- 10
 - Duration: 21 days
 - Temperature: 37°C ± 2°C
 - Stress condition: During storage, samples were invented 360° to simulate patient use out of refrigerated storage. The rotations were performed 100 times, five days every week.

- 15 The number of sub-visible particles was quantified as described in example 5.

Table 13 The effect of buffer substance on co-formulation physical stability

Co-formulation	Buffer substance	Number of sub-visible particles as a function of time (days)					
		0	7	11	14	18	21

Particle size >5µm							
1	Citrate	459	668	408	409	4717	3115
14	Histidine	386	752	569	571	2495	2392
Particle size >10µm							
1	Citrate	52	117	55	125	2114	1446
14	Histidine	68	80	103	151	969	760
Particle size >25µm							
1	Citrate	0	0	0	16	542	332
14	Histidine	2	0	4	13	162	117

Results are the mean of 2 replicates and has been rounded to nearest integer value

Concluding Remarks

5 Until day 14 of having been stored at stressed conditions, the physical stability of the two co-formulations was similar and acceptable. However, after 18 days, the number of sub-visible particles in the citrate-buffered co-formulation (co-formulation 1) was much greater than that in the histidine-buffered co-formulation (co-formulation 14). The histidine-buffered co-formulation 14 was the most stable.

10 **EXAMPLE 8: EFFECT OF DIFFERENT BUFFER CONCENTRATIONS ON CO-FORMULATION CHEMICAL STABILITY**

 This example shows the effect of buffer concentration on the chemical stability of otherwise identical co-formulations.

Composition

15 The compositions of co-formulation containing different concentrations of buffer are shown in table 14.

Table 14 Composition of co-formulations containing different buffer concentrations

Ingredient	Co-formulation	
	15	16
Cagrilintide drug substance (mg/ml)	3.2	3.2
Semaglutide drug substance (mg/ml)	3.2	3.2
HP-B-CD	10	10

KLEPTOSE® (Roquette) (% w/v) (Average MS: 0.62, MS range: 0.58-0.68) ¹		
L-Histidine (mM)	6	20
HCl	q.s.	q.s.
NaOH	q.s.	q.s.
WFI	To make 1 ml	To make 1 ml
pH	5.7	5.7

¹ MS: molar substitution, corresponds to hydroxypropyls per glucose unit

Preparation process

Formulations were prepared as described in example 1.

Methods

Samples were stored at 30°C for 21 days and analysed to determine the chemical purity of cagrilintide after 7, 14, and 21 days. Purity of cagrilintide was determined as described in example 4 (for semaglutide).

Table 15 The effect of buffer concentration on cagrilintide chemical stability in co-formulation

Co-formulation	Buffer concentration	Cagrilintide purity (%) as a function of time (days) at 30°C			
		0	7	14	21
15	Histidine: 6 mM	89.2%	88.2%	86.5%	84.2%
16	Histidine: 20 mM	89.2%	87.9%	86.0%	83.5%

Concluding Remarks

The results in table 15 show that both co-formulations were stable. However, the chemical purity of cagrilintide was highest in co-formulation 15. The purity of cagrilintide decreased more rapidly over time when the histidine concentration was 20mM.

EXAMPLE 9: EFFECT OF DIFFERENT BUFFER CONCENTRATIONS ON CO-FORMULATION PHYSICAL STABILITY

This example shows the effect of histidine buffer concentration on co-formulation physical stability.

Composition

The compositions of the tested co-formulations are as shown in table 14.

5 Preparation process

Formulations were prepared as described in example 1.

Methods

All samples were stored for at stressed condition defined as:

- 10
- Duration: 18 days
 - Temperature: 37°C ± 2°C
 - Stress condition: During storage, samples were invented 360° to simulate patient use out of refrigerated storage. The rotations were performed 100 times, five days every week.

15

The number of sub-visible particles was quantified as described in example 5.

Table 16 The effect of buffer concentration on co-formulation physical stability

Co- formulation	Buffer concentration	Number of sub-visible particles as a function of time point (days)				
		0	7	10	14	18
Particle size >5µm						
15	Histidine: 6 mM	239	392	165	189	266
16	Histidine: 20 mM	50	262	197	1029	926
Particle size >10µm						
15	Histidine: 6mM	4	8	15	17	46
16	Histidine: 20 mM	2	17	17	461	423
Particle size >25µm						
15	Histidine: 6mM	0	0	2	0	2
16	Histidine: 20 mM	0	0	0	120	104

Results are the mean of 2 replicates and has been rounded to nearest integer value.

Concluding Remarks

20

The difference in the physical stability of co-formulations 15 and 16 became most apparent after 14 days. The data in table 16 show that the number of sub-visible particles seen in co-formulation 16 (containing 20 mM histidine) was greater than the number of sub-

visible particles seen in co-formulation 15 (containing 6 mM histidine). That is, the co-formulation comprising 6 mM histidine was the most physically stable.

EXAMPLE 10: EFFECT OF HP-B-CD CONCENTRATION ON SUBCUTANEOUS
5 TOLERANCE UPON SUBCUTANEOUS INJECTION

This example shows the concentration dependent effect of HP-B-CD on the subcutaneous tissue upon subcutaneous injection.

Composition

10 The compositions of the tested co-formulation vehicles prepared with varying HP-B-CD concentrations are shown in table 17.

Table 17 Composition of co-formulation vehicles containing varying concentrations of HP-B-CD

Ingredient	Co-formulation vehicle				
	1	2	3	4	5
HP-B-CD KLEPTOSE® (Roquette) (% w/v) (Average MS: 0.62, MS range: 0.58-0.68) ¹	10	12.5	15	17.5	20
L-Histidine (mM)	6	6	6	6	6
Sorbitol ² (mg/ml)	28	24	20	16	12
Polysorbate 80 (mg/ml)	0.05	0.05	0.05	0.05	0.05
HCl	q.s.	q.s.	q.s.	q.s.	q.s.
NaOH	q.s.	q.s.	q.s.	q.s.	q.s.
WFI	To 1 ml	To 1 ml	To 1 ml	To 1 ml	To 1 ml
pH	6.0	6.0	6.0	6.0	6.0

15 ¹ MS: molar substitution, corresponds to hydroxypropyls per glucose unit.
² Sorbitol concentration varies with varying HP-B-CD concentration to maintain isotonic conditions.

Preparation process

Formulations were prepared as described in example 1 except that addition of active pharmaceutical ingredients was abstained from.

Method

5 The local (subcutaneous) tolerance upon subcutaneous administration of formulations containing HP-B-CD was studied in 5 live Landrace×Yorkshire×Duroc (LYD) pigs by evaluation of the resulting skin lesions 6 days (necropsy) after subcutaneous administration of 600µl using syringes equipped with 25 G sized needles and 5mm stoppers. Skin samples sized 2x2cm were collected at necropsy, fixed in neutral buffered formalin, 10 trimmed using multi-knife, embedded in paraffin, cut in 4 µm thin sections, mounted on glass slides and subsequently hematoxylin-eosin (HE) stained. The level of subcutaneous tissue necrosis was assessed using a light microscope and scored on a numerical scale, where code 1 reflects ‘no necrosis’ and code 4 reflects ‘moderate necrosis’. For each co-formulation vehicle, a total of 5 skin samples were performed. However, due to variation in slicing the 15 subcutaneous tissue for successful evaluation of the necrosis, not all injection sites could be assigned a score:

1, no necrosis
2, minimal necrosis
3, mild necrosis
20 4, moderate necrosis

Isotonic co-formulation vehicle preparations containing 10% w/v to 20% w/v HP-B-CD were evaluated for the level of subcutaneous necrosis that they elicited upon subcutaneous injection. The results are presented in table 20.

25 **Table 18 Necrosis scores for subcutaneous tissue necrosis seen 6 days after the injection of co-formulation vehicles varying in percentage HP-B-CD**

Co-formulation vehicle	HP-B-CD concentration	Necrosis, subcutaneous	Number of successfully scored skin samples of total
1	10% w/v	2,2,2,2,3	5 of 5
2	12.5% w/v	3,3	2 of 5
3	15% w/v	2,2,3	3 of 5
4	17.5% w/v	2,2,3	3 of 5

5	20% w/v	2,3,3,4	4 of 5
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Concluding Remarks

A correlation was observed between increased HP-B-CD concentration in the co-formulation vehicle and necrosis at the injection site. In one case, the co-formulation vehicle containing 20% w/v HP-B-CD gave rise to moderate subcutaneous necrosis at the injection site. Formulations containing less than 20% w/v HP-B-CD all gave rise to only mild or minimal subcutaneous necrosis at the injection site. All co-formulation vehicles containing 10-20% w/v HP-B-CD were tolerated to an acceptable degree, those containing 10-17.5% w/v HP-B-CD being preferred.

EXAMPLE 11: EFFECT OF DIFFERENT TONICITY AGENTS ON SUBCUTANEOUS TOLERANCE UPON SUBCUTANEOUS INJECTION

This example shows the effect on local tolerance of any one of three different tonicity agents (sorbitol, mannitol and trehalose) in otherwise identical isotonic co-formulation vehicles.

Composition

The compositions of the tested co-formulation vehicles are shown in table 19.

Table 19 Composition of isotonic co-formulation vehicles prepared with difference tonicity agents

Ingredient	Co-formulation vehicle		
	6	7	8
HP-B-CD KLEPTOSE® (Roquette) (% w/v) (Average MS: 0.62, MS range: 0.58-0.68) ¹	15	15	15
L-Histidine (mM)	6	6	6
Polysorbate 20 (mg/ml)	0.1	0.1	0.1
Sorbitol (mg/ml)	24	-	-
Mannitol (mg/ml)	-	25	-
Trehalose (mg/ml)	-	-	46
HCl	q.s.	q.s.	q.s.

NaOH	q.s.	q.s.	q.s.
WFI	To make 1ml	To make 1ml	To make 1ml
pH	6.0	6.0	6.0

¹MS: molar substitution, corresponds to hydroxypropyls per glucose unit

Preparation process

Formulations were prepared as described in example 1 except that addition of active pharmaceutical ingredients was abstained from.

Method

The local tolerance from subcutaneous administration of isotonic vehicle preparations containing HP-B-CD and three different tonicity agents was studied in 2 live Landrace×Yorkshire×Duroc (LYD) pigs by evaluation of the skin reactions resulting from subcutaneous administration of 600 µl vehicle preparation. The preparations were injected using syringes equipped with 25 G sized needles and 5mm stoppers. Approx. 24 hours post injection necropsy was performed and skin samples sized 2x2cm were fixed in neutral buffered formalin and trimmed into 4µm sections using multi-knife, embedded in paraffin and subsequently HE stained. For the two samples, the severity of the subcutaneous tissue necrosis, inflammatory cell infiltration and haemorrhage distribution was assessed by a trained toxicopathologist using a light microscope and scored on a numerical scale, where code 1 reflects ‘no abnormality’ and code 3 reflects ‘mild severity’:

- 1, no abnormality
- 2, minimal severity
- 3, mild severity

Table 20 Severity scores 24 hours post subcutaneous injections of subcutaneous tissue necrosis, inflammatory cell infiltration and haemorrhage distribution for co-formulation vehicles containing three types of tonicity agents

Co-formulation vehicle	Tonicity agent	Necrosis	Inflammatory cell infiltration	Haemorrhage distribution
6	Sorbitol	2,1	2,1	1,1
7	Mannitol	2,2	2,3	1,2
8	Trehalose	1,1	2,2	1,2

Concluding Remarks

5 The data presented in table 20 show that, overall, sorbitol was the tonicity agent that resulted in the least severe necrosis, inflammatory cell infiltration and haemorrhage. These observations confirm that sorbitol is the preferred tonicity agent for obtaining good and acceptable subcutaneous tolerability of the co-formulation containing the active pharmaceutical ingredients.

10 **EXAMPLE 12: CONFIRMATION ON THE EFFECT OF TONICITY AGENT TYPE IN THE HISTIDINE-BUFFERED FORMULATION AND THE EFFECT OF THE CITRATE-BUFFERED FORMULATION ON THE SUBCUTANEOUS TOLERANCE UPON SUBCUTANEOUS INJECTION**

 This experiment examines:

- 15 (1) the effect that the type of tonicity agent has on the local tolerance profile, upon subcutaneous injection, of an otherwise identical, histidine-buffered co-formulation; and
 (2) the effect on the local tolerance profile, upon subcutaneous injection, of a co-formulation vehicle with a citrate-buffered formulation and no tonicity agent.

20 **Composition**

 The compositions of the evaluated co-formulations are described in table 21 and 22.

Table 21 Composition of isotonic histidine-buffered co-formulations

Ingredient	Co-formulation	
	17	18
Cagrilintide drug substance (mg/ml)	3.2	3.2
Semaglutide drug substance (mg/ml)	3.2	3.2
HP-B-CD KLEPTOSE® (Roquette) (% w/v) (Average MS: 0.62, MS range: 0.58-0.68) ¹	15	15
L-Histidine (mM)	6	6
Trehalose (mg/ml)	38.6	-
Sorbitol (mg/ml)	-	20
Polysorbate 80 (mg/ml)	0.05	0.05

HCl	q.s.	q.s.
NaOH	q.s.	q.s.
WFI	To 1 ml	To 1 ml
pH	6.0	6.0

¹ MS: molar substitution, corresponds to hydroxypropyls per glucose unit

Table 22 Composition of isotonic citrate-buffered co-formulation vehicle

Ingredient	Co-formulation vehicle
	9
HP-B-CD KLEPTOSE® (Roquette) (% w/v) (Average MS: 0.92, MS range: 0.81-0.99) ¹	25
Citrate, 1 H ₂ O (mM)	3
HCl	q.s.
NaOH	q.s.
WFI	To 1 ml
pH	5.7

¹ MS: molar substitution, corresponds to hydroxypropyls per glucose unit

5 Preparation process

Co-formulations 17 and 18 were prepared as described in example 1. Co-formulation vehicle 9 was prepared as described in example 1 except that addition of active pharmaceutical ingredients was abstained from.

10 Method

The local tolerance upon subcutaneous administration of the co-formulations described in table 23a and 23b was studied in 8 live minipigs, by evaluation of the resulting skin lesions 6 days (necropsy) after subcutaneous administration of sample sizes of 750µl using syringes equipped with 25 G sized needles and 5mm stoppers. Skin samples sized 2x2cm were collected at necropsy, fixed in neutral buffered formalin, trimmed using multi-knife, embedded in paraffin, cut in 4 µm thin sections, mounted to glass slides and subsequently hematoxylin-eosin (HE) stained. For the samples, the severity of the subcutaneous tissue necrosis was assessed by a trained toxicopathologist using a light microscope and scored on a numerical scale, where code 1 reflects ‘no abnormalities’ and code 5 reflects ‘marked severity’:

- 1, no abnormalities
- 2, minimal severity
- 3, mild severity
- 4, moderate severity
- 5, marked severity

Results on scores of necrosis are shown in table 23 and table 24.

Table 23 **Severity scores 6 days post injections of subcutaneous tissue necrosis for histidine-buffered co-formulations**

Co-formulation	Buffer	Tonicity agent	Necrosis scores for four skin samples
17	Histidine	Trehalose	1,2,3,3
18	Histidine	Sorbitol	2,2,2,2

Table 24 **Severity scores 6 days post injections of subcutaneous tissue necrosis for citrate-buffered co-formulation vehicle**

Co-formulation vehicle	Buffer	Tonicity agent	Necrosis scores for eight skin samples
9	Citrate	None	3,4,4,4,4,5,5,5

Concluding Remarks

The results presented in table 23 show that the type of tonicity agent included in the formulation affects its *in vivo* local tolerability. There is a correlation between tonicity agent and observed subcutaneous necrosis at the injection site. The subcutaneous injection of co-formulation 17, comprising trehalose, resulted in two events of mild necrosis (a score of 3). The subcutaneous injection of co-formulation 18, comprising sorbitol, resulted in minimal necrosis only (a score of 2), which is a better outcome. These results confirm that, in the case of this otherwise identical co-formulation vehicle, the co-formulation comprising 15% w/v HP-B-CD (Average MS: 0.62) and sorbitol is better than that comprising 15% w/v HP-B-CD (Average MS: 0.62) and trehalose.

The results presented in table 24 show that three events of marked necrosis (a score of 5) were observed with co-formulation 9 vehicle comprising 25% w/v HP-B-CD (Average MS: 0.92), citrate and no tonicity agent, confirming the unsuitability of this particular co-formulation for subcutaneous administration.

EXAMPLE 13: EFFECT OF HYDROXYPROPYL-SUBSTITUTED CYCLODEXTRINS OF VARYING TYPE ON CAGRILINTIDE AND SEMAGLUTIDE CO-FORMULATION PHYSICAL AND CHEMICAL STABILITY

5 This example shows the effect of hydroxypropyl-alpha-cyclodextrin (HP-A-CD), hydroxypropyl-beta-cyclodextrin (HP-B-CD) and hydroxypropyl-gamma-cyclodextrin (HP-G-CD) on the formation of sub-visible particles and chemical degradation of cagrilintide in an otherwise identical cagrilintide and semaglutide co-formulation.

Composition

10 The compositions of co-formulation 20, 21 and 23 are shown in table 25.

Table 25 Composition of co-formulations with hydroxypropyl cyclodextrins of varying type

Ingredient	Co-formulation		
	20	21	22
Cagrilintide drug substance (mg/ml)	3.2	3.2	3.2
Semaglutide drug substance (mg/ml)	3.2	3.2	3.2
HP-A-CD (CycloLab) (% w/v) (Average MS: 0.8, MS range: 0.5-0.9) ¹	15	-	-
HP-B-CD KLEPTOSE® (Roquette) (% w/v) (Average MS: 0.62, MS range: 0.58-0.68) ¹	-	15	-
HP-G-CD (CycloLab) (% w/v) (Average MS: 0.6, MS range: 0.4-0.7) ¹	-	-	15
L-Histidine (mM)	6	6	6
Sorbitol (mg/ml)	20	20	20
Polysorbate 80 (mg/ml)	0.05	0.05	0.05
HCl	q.s.	q.s.	q.s.
NaOH	q.s.	q.s.	q.s.
WFI	To make 1 ml	To make 1 ml	To make 1 ml
pH	5.8	5.8	5.8

¹ MS: molar substitution, corresponds to hydroxypropyls per glucose unit

Preparation process

Formulations were prepared as described in example 1.

5 Methods

Samples used to determine sub-visible particle count were stored at stressed conditions, defined as:

- Duration: 42 days
- Temperature: 30°C ± 2°C
- Stress condition: During storage, samples were inverted 360° to simulate patient use out of refrigerated storage. The rotations were performed 20 times three days every week, and 40 times two days every week.

The number of sub-visible particles was determined as described in example 5.

Samples used to determine the purity of cagrilintide were stored at 37°C for up to 42 days. Purity of cagrilintide was determined using the following reversed phase high performance liquid chromatography (RP-HPLC) where the samples were analysed using a Kinetex C18, 2.6 µm column (4.6 x 150 mm) with a gradient elution of eluent A consisting of 90% v/v 0.09 M phosphate solution, pH 3.6 and 10% v/v acetonitrile, and eluent B consisting of 60% v/v acetonitrile and 20% v/v isopropanol. Chromatography was conducted with UV detection (210 nm) at 30°C using a 10-100 µl injection volume and a flow rate of 0.7 ml/min. Purity of cagrilintide was quantified as being the area of the main peak divided by the area of all related peaks x 100%.

The same method was used to determine the purity of semaglutide in other experiments.

Table 26 Physical stability of cagrilintide and semaglutide co-formulations formulated with hydroxypropyl cyclodextrins of varying type

Co-formulation	Type of hydroxypropyl substituted cyclodextrin	Number of sub-visible particles as a function of time (days)					
		0	14	21	28	35	42
		Particle size >5µm					
20	HP-A-CD	65	196	270	441	523	690
21	HP-B-CD	49	122	61	245	324	503

22	HP-G-CD ¹	453537	-	-	-	-	-
Particle size >10µm							
20	HP-A-CD	4	7	14	27	47	41
21	HP-B-CD	4	8	5	20	20	116
22	HP-G-CD ¹	107013	-	-	-	-	-
Particle size >25µm							
20	HP-A-CD	0	0	0	1	3	0
21	HP-B-CD	0	1	1	1	4	15
22	HP-G-CD ¹	316	-	-	-	-	-

Results for number of sub-visible particles are the mean of 3 replicates and has been rounded to nearest integer value

(-) Sampling not performed

¹ For co-formulation 22 with HP-G-CD, sampling was discontinued earlier than for the other formulations because of rapid increases in particle counts.

Table 27 Chemical purity (%) of cagrilintide in cagrilintide and semaglutide co-formulations formulated with hydroxypropyl cyclodextrins of varying type

Co-formulation	Type of hydroxypropyl cyclodextrin	Cagrilintide purity (%) as a function of time (days) at 37°C			
		0	14	28	42
20	HP-A-CD	94.0%	87.2%	79.0%	71.1%
21	HP-B-CD	96.8%	88.9%	81.9%	75.0%

Concluding Remarks

The results presented in table 26 show that in co-formulation 22 (HP-G-CD), high numbers of sub-visible particles were observed already at time zero, which preclude the use of HP-G-CD to co-formulate cagrilintide and semaglutide. The sampling for the analysis of sub-visible particle counts was discontinued for co-formulation 22 containing HP-G-CD after the initial analysis at time zero. For co-formulation 20 (HP-A-CD) and co-formulation 21 (HP-B-CD) virtually no increase in the number of sub-visible particles was observed.

The results presented in table 27 for the chemical purity of cagrilintide with either HP-A-CD or HP-B-CD, show a slightly more rapid decrease in cagrilintide purity in co-formulation 20, containing HP-A-CD, than in co-formulation 21 containing HP-B-CD.

Based on results in table 26, either HP-A-CD or HP-B-CD is acceptable for co-formulations of cagrilintide and semaglutide. However, based on results in table 27, HP-B-CD is preferred compared to HP-A-CD for a cagrilintide and semaglutide co-formulation, due to the superior purity of cagrilintide when formulated with HP-B-CD.

5

EXAMPLE 14: EFFECT OF TYPE OF BETA-CYCLODEXTRIN SUBSTITUTION ON THE PHYSICAL STABILITY OF THE CO-FORMULATION

This example shows the effect of sulfobutylether-B-cyclodextrin (SBE-B-CD) and hydroxypropyl-beta-cyclodextrin on the physical stability of otherwise identical cagrilintide and semaglutide co-formulations.

10

Composition

The compositions of co-formulations containing either HP-B-CD or SBE-B-CD are shown in table 28.

15

Table 28 Composition of co-formulation containing either HP-B-CD or SBE-B-CD

Ingredient	Co-formulation	
	23	24
Cagrilintide drug substance (mg/ml)	3.2	3.2
Semaglutide drug substance (mg/ml)	3.2	3.2
HP-B-CD KLEPTOSE® (Roquette) (% w/v) (Average MS: 0.62, MS range: 0.58-0.68) ¹	15	-
SBE-B-CD (CycloLab) (%w/v) (Average MS: 0.87, MS range: 0.84-0.99) ¹	-	15
L-Histidine (mM)	6	6
Sorbitol (mg/ml)	20	20
Polysorbate 80 (mg/ml)	0.05	0.05
HCl	q.s.	q.s.
NaOH	q.s.	q.s.

WFI	To 1 ml	To 1 ml
pH	5.8	5.8

¹ MS: molar substitution, corresponds to sulfobutyl ethers/hydroxypropyls per glucose unit

Preparation process

Formulations were prepared as described in example 1.

5

Methods

Samples used to determine the number of sub-visible particles were stored at stressed conditions, defined as:

- Duration: 35 days
- 10 - Temperature: 30°C ± 2°C
- Stress condition: During storage, samples were inverted 360° to simulate patient use out of refrigerated storage. The rotations were performed 20 times three days every week, and 40 times two days every week.

The number of sub-visible particles was quantified as described in example 5.

15

Table 29 Levels of sub-visible particles in cagrilintide and semaglutide co-formulations containing either HP-B-CD or SBE-B-CD

Co- formulation	Type of substitution	Number of sub-visible particles as a function of time (days)				
		0	14	21	28	35
Particle size >5µm						
23	HP-B-CD	49	122	61	245	324
24	SBE-B-CD	317	1686	6940	20714	280478
Particle size >10µm						
23	HP-B-CD	4	8	5	20	20
24	SBE-B-CD	13	640	3503	10248	138324
Particle size >25µm						
23	HP-B-CD	0	1	1	1	4
24	SBE-B-CD	0	126	738	2008	29834

Results for number of sub-visible particles are the mean of 3 replicates and has been rounded to nearest integer value

20

Concluding Remarks

The results in table 29 show that when using SBE-B-CD to co-formulate cagrilintide and semaglutide, a large increase in the number of sub-visible particles is observed after 14 days; that is, the co-formulation is physically unstable. When using a HP-B-CD instead, virtually no increase is observed during the 35 days duration of the study; that is, the co-formulation is physically stable.

In contrast to what we have shown for hydroxypropyl-beta-cyclodextrin, these results demonstrate that sulfobutylether-B-cyclodextrin (SBE-B-CD) is not a suitable cyclodextrin to use for co-formulating cagrilintide and semaglutide.

EXAMPLE 15: EFFECT OF CAGRILINTIDE AND SEMAGLUTIDE CONCENTRATION RATIOS ON THE PHYSICAL STABILITY OF THE CO-FORMULATION

This example shows the effect of different concentration ratios of cagrilintide and semaglutide on the levels of sub-visible particles observed in the co-formulation.

Composition

The composition of histidine-buffered co-formulations 25 to 29 are shown in table 30, and the composition of histidine-buffered co-formulations 30 to 40 are shown in table 31.

Table 30 Composition of histidine-buffered co-formulations with varying cagrilintide and semaglutide concentration ratios

Ingredient	Co-formulation				
	25	26	27	28	29
Cagrilintide drug substance (mg/ml)	3.2	3.2	3.2	3.2	3.2
Semaglutide drug substance (mg/ml)	3.2	4.8	6.4	8.0	9.6
HP-B-CD KLEPTOSE® (Roquette) (% w/v) (Average MS: 0.62, MS range: 0.58-0.68) ¹	15	15	15	15	15
L-Histidine (mM)	6	6	6	6	6
Sorbitol (mg/ml)	20	20	20	20	20

Polysorbate 80 (mg/ml)	0.05	0.05	0.05	0.05	0.05
HCl	q.s.	q.s.	q.s.	q.s.	q.s.
NaOH	q.s.	q.s.	q.s.	q.s.	q.s.
WFI	To 1 ml	To 1 ml	To 1 ml	To 1 ml	To 1 ml
pH	5.8	5.8	5.8	5.8	5.8

¹ MS: molar substitution, corresponds to hydroxypropyls per glucose unit

Table 31 **Composition of histidine-buffered co-formulations with modified composition with varying cagrilintide and semaglutide concentration ratios**

[illegible]

WFI	To 1 ml	To 1 ml	To 1 ml	To 1 ml	To 1 ml	To 1 ml	To 1 ml	To 1 ml	To 1 ml	To 1 ml	To 1 ml
pH	5.8	5.8	5.8	5.8	5.8	5.8	5.8	5.8	5.8	5.8	5.8

¹ MS: molar substitution, corresponds to hydroxypropyls per glucose unit

Preparation process

Formulations were prepared as described in example 1.

5

Methods

Samples used to determine the number of sub-visible particles were stored at stressed conditions, defined as:

- Duration: 28 days
- 10 - Temperature: 30°C ± 2°C
- Stress condition: During storage, samples were inverted 360° to simulate patient use out of refrigerated storage. The rotations were performed 20 times three days every week, and 40 times two days every week.

The number of sub-visible particles was quantified as described in example 5.

15

Table 32 Levels of sub-visible particles in the co-formulation containing different concentration ratios of cagrilintide and semaglutide

Co- formulation	Cagrilintide / semaglutide concentration (ratio)	Number of sub-visible particles as a function of time (days)			
		0	14	21	28
Particle size >5µm					
25	3.2 / 3.2 mg/ml (1:1)	207	214	542	341
26	3.2 / 4.8 mg/ml (1:1.5)	65	133	214	591
27	3.2 / 6.4 mg/ml (1:2)	55	76	198	134
28	3.2 / 8.0 mg/ml (1:2.5)	44	86	262	139
29	3.2 / 9.6 mg/ml (1:3)	56	184	137	540
30	3.2 / 3.2 mg/ml (1:1)	297	467	650	11684
31	3.2 / 4.8 mg/ml (1:1.5)	377	541	331	14453
32	3.2 / 6.4 mg/ml (1:2)	588	501	980	45916
33	3.2 / 8.0 mg/ml (1:2.5)	452	341	712	13108
34	3.2 / 9.6 mg/ml (1:3)	37	226	121	161
35	3.2 / 9.6 mg/ml (1:3)	55	77	290	315
36	3.2 / 9.6 mg/ml (1:3)	41	181	273	368
37	3.2 / 9.6 mg/ml (1:3)	312	457	445	25494
38	3.2 / 10.7 mg/ml (1:3.33)	1240	594	1043	53301
39	3.2 / 12.8 mg/ml (1:4)	1459	549	320	77697
40	3.2 / 16 mg/ml (1:5)	1025	568	877	5202
Particle size >10µm					
25	3.2 / 3.2 mg/ml (1:1)	18	31	25	51
26	3.2 / 4.8 mg/ml (1:1.5)	5	9	22	106
27	3.2 / 6.4 mg/ml (1:2)	3	9	18	24
28	3.2 / 8.0 mg/ml (1:2.5)	10	9	13	13
29	3.2 / 9.6 mg/ml (1:3)	1	9	8	130
30	3.2 / 3.2 mg/ml (1:1)	38	45	79	1146
31	3.2 / 4.8 mg/ml (1:1.5)	69	94	80	2053
32	3.2 / 6.4 mg/ml (1:2)	74	81	171	7608
33	3.2 / 8.0 mg/ml (1:2.5)	78	57	118	1651
34	3.2 / 9.6 mg/ml (1:3)	5	9	5	14
35	3.2 / 9.6 mg/ml (1:3)	4	8	22	59

36	3.2 / 9.6 mg/ml (1:3)	0	15	27	61
37	3.2 / 9.6 mg/ml (1:3)	50	79	74	3233
38	3.2 / 10.7 mg/ml (1:3.33)	147	72	165	6793
39	3.2 / 12.8 mg/ml (1:4)	155	84	51	10270
40	3.2 / 16 mg/ml (1:5)	80	99	189	382
Particle size >25µm					
25	3.2 / 3.2 mg/ml (1:1)	1	0	4	1
26	3.2 / 4.8 mg/ml (1:1.5)	0	1	0	6
27	3.2 / 6.4 mg/ml (1:2)	0	1	3	3
28	3.2 / 8.0 mg/ml (1:2.5)	1	0	3	3
29	3.2 / 9.6 mg/ml (1:3)	0	1	0	9
30	3.2 / 3.2 mg/ml (1:1)	0	0	1	13
31	3.2 / 4.8 mg/ml (1:1.5)	4	0	3	60
32	3.2 / 6.4 mg/ml (1:2)	8	4	6	157
33	3.2 / 8.0 mg/ml (1:2.5)	8	3	3	25
34	3.2 / 9.6 mg/ml (1:3)	0	0	1	0
35	3.2 / 9.6 mg/ml (1:3)	3	0	0	3
36	3.2 / 9.6 mg/ml (1:3)	0	0	4	0
37	3.2 / 9.6 mg/ml (1:3)	1	5	0	42
38	3.2 / 10.7 mg/ml (1:3.33)	1	0	5	97
39	3.2 / 12.8 mg/ml (1:4)	3	3	1	68
40	3.2 / 16 mg/ml (1:5)	8	4	26	8

Results for number of sub-visible particles are the mean of 3 replicates and has been rounded to nearest integer value

Concluding Remarks

5 The results presented in tables 32 show that after 21 days, virtually no increase in sub-visible particle count was seen in co-formulation 25-39 containing 3.2 mg/ml cagrilintide and up to 12 mg/ml semaglutide.

 After 14, an increase in sub-visible particle count was seen for co-formulation 40 containing 3.2 mg/ml cagrilintide and 16 mg/ml semaglutide.

10 All histidine-buffered co-formulations 25 to 40 comprising 3.2 mg/ml cagrilintide and up to 16 mg/ml semaglutide was physically stable.

EXAMPLE 16: EFFECT OF CONCENTRATION OF HP-B-CD ON THE PHYSICAL STABILITY OF THE CO-FORMULATION

5 This example shows the effect of HP-B-CD concentration on the physical stability of the cagrilintide and semaglutide co-formulation, when the co-formulation is exposed to physical stress.

Composition

10 The composition of co-formulation 41 to 44 with the histidine-buffered composition is shown in table 32.

Table 32 Composition of co-formulation with varying HP-B-CD concentrations

Ingredient	Co-formulation			
	41	42	43	44
Cagrilintide drug substance (mg/ml)	3.2	3.2	3.2	3.2
Semaglutide drug substance (mg/ml)	3.2	3.2	3.2	3.2
HP-B-CD KLEPTOSE® (Roquette) (% w/v) (Average MS: 0.62, MS range: 0.58-0.68) ¹	7.5	10	12.5	15
L-Histidine (mM)	6	6	6	6
Sorbitol (mg/ml)	20	20	20	20
Polysorbate 80 (mg/ml)	0.05	0.05	0.05	0.05
HCl	q.s.	q.s.	q.s.	q.s.
NaOH	q.s.	q.s.	q.s.	q.s.
WFI	To 1 ml	To 1 ml	To 1 ml	To 1 ml
pH	5.8	5.8	5.8	5.8

¹ MS: molar substitution, corresponds to hydroxypropyls per glucose unit

Preparation process

15 Formulations were prepared as described in example 1.

Method

20 The propensity of cagrilintide and semaglutide in the co-formulation to aggregate and form peptide fibrils was measured using a Thioflavin T (ThT) fluorescence stress assay as described in example 2.

Table 33 Physical stability for cagrilintide and semaglutide co-formulation with varying HP-B-CD concentrations

Co-formulation	HP-B-CD (MS: 0.62) ²	Lag time until fibrillation
41	7.5% w/v	17.66 hours ¹
42	10% w/v	28.06 hours ¹
43	12.5% w/v	70.97 hours ¹
44	15% w/v	119.0 hours ¹

¹ Result is the mean of 6 replicates

5 ² MS: molar substitution, corresponds to hydroxypropyls per glucose unit

Concluding Remarks

10 The results presented in table 33 show that the physical stability of the cagrilintide and semaglutide co-formulation is dependent upon the concentration of HP-B-CD, with lower concentrations resulting in shorter lag time until fibrillation occurs. The co-formulation comprising 7.5% w/v HP-B-CD was the least stable. The co-formulation comprising 15% w/v HP-B-CD was the most stable.

15 **EXAMPLE 17: LOCAL TOLERANCE, IN PIGS, OF SUBCUTANEOUSLY INJECTED VEHICLE FORMULATIONS VARYING IN HP-B-CD CONTENT AND MOLAR SUBSTITUTION DEGREE, AS WELL AS IN OVERALL BUFFER COMPOSITION**

 This experiment examined:

- (1) the effect that HP-B-CD concentration and average MS (0.62 versus 0.92) had on the local tolerance profile, upon subcutaneous injection.
- 20 (2) the effect that the formulation vehicle has on the local tolerance profile in the presence of HP-B-CD, upon subcutaneous injection.

Composition

 The compositions of the tested co-formulation vehicles are shown in table 34.

25 **Table 34 Composition of co-formulation vehicles varying in HP-B-CD content and molar substitution degree as well as in overall buffer composition**

Ingredient	Co-formulation vehicles									
	10	11	12	13	14	15	16	17	18	19
HP-B-CD	15	20	22	22	25	20	22	-	15	20

KLEPTOSE® (Roquette) (% w/v) (Average MS: 0.62, MS range: 0.58- 0.68) ¹										
HP-B-CD KLEPTOSE® (Roquette) (% w/v) (Average MS: 0.92, MS range: 0.81- 0.99) ¹	-	-	-	-	-	-	-	22	-	-
L-Histidine (mM)	6	6	6	6	6	-	-	-	6	6
Citrate, 1 H ₂ O (mM)	-	-	-	-	-	3	3	3	-	-
Sorbitol (mg/ml)	20	20	20	35	20	-	-	-	20	20
Polysorbate 80 (mg/ml)	0.05	0.05	0.05	0.05	0.05	-	-	-	0.05	0.05
m-cresol (mg/ml)	-	-	-	-	-	-	-	-	2.70 (25mM)	2.70 (25mM)
Phenol (mg/ml)	-	-	-	-	-	-	-	-	2.35 (25mM)	2.35 (25mM)
EDTA disodium, dihydrate (mg/ml)	-	-	-	-	-	-	-	-	2.0	2.0
HCl	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.
NaOH	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.
WFI	To 1 ml	To 1 ml	To 1 ml	To 1 ml	To 1 ml	To 1 ml	To 1 ml	To 1 ml	To 1 ml	To 1 ml
pH	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0

¹ MS: molar substitution, corresponds to hydroxypropyls per glucose unit

Preparation process

- Formulations were prepared as described in example 1 except that active
5 pharmaceutical ingredients were not added.

Method

The local (subcutaneous) tolerance, upon subcutaneous administration, of formulations containing HP-B-CD was studied in 4 live Landrace×Yorkshire×Duroc (LYD) pigs by evaluation of the resulting skin lesions 5 days (necropsy) after subcutaneous administration of 200 µl using NovoPen 4 with NovoFine Plus needles (32 G/4 mm). Skin samples sized 2x2 cm were collected at necropsy, fixed in neutral buffered formalin, trimmed using multi-knife, embedded in paraffin, cut in 4 µm thin sections, mounted on glass slides and subsequently hematoxylin-eosin (HE) stained. For the four samples, the severity of the subcutaneous tissue necrosis and inflammatory cell infiltration was assessed by a trained toxicopathologist using a light microscope and scored on a numerical scale, where code 1 reflects ‘no abnormality detected’ and code 5 reflects ‘marked severity’:

- 1, no abnormality detected
- 2, minimal severity
- 3, mild severity
- 4, moderate severity
- 5, marked severity

The co-formulation vehicles were evaluated for the level of subcutaneous tissue necrosis and inflammatory cell infiltration that they elicited upon subcutaneous injection. The results are presented in table 35.

20

Table 35 Severity scores: severity of subcutaneous tissue necrosis and inflammatory cell infiltration 5 days post injection, for co-formulation vehicles varying in HP-B-CD content and molar substitution degree as well as in overall buffer composition

Co-formulation vehicle	Necrosis	Inflammatory cell infiltration, granulomatous
10	1, 1, 1, 1	2, 2, 2, 2
11	1, 1, 2, 2	2, 2, 2, 3
12	2, 3, 4, 4	3, 3, 4, 4
13	2, 4, 4, 4	3, 3, 4, 4
14	3, 4, 4, 4	3, 3, 4, 5
15	1, 3, 3, 3	1, 3, 3, 3
16	3, 4, 5, 5	4, 4, 4, 5

17	1,5,4,5	2,4,4,5
18	1,2,2,2	2,2,3,3
19	1,1,2,2	2,2,3,3

Concluding Remarks

The results in table 35 show that the *in vivo* local subcutaneous tolerability depended on the concentration of HP-B-CD and the overall buffer composition. Co-
5 formulation vehicles comprising histidine and sorbitol showed better tolerability than those comprising citrate.

Co-formulation vehicles comprising 20% w/v HP-B-CD or less resulted mainly in no or minimal necrosis or inflammatory cell infiltration (scores of 1 or 2) and a single observation of mild inflammatory cell infiltration (a score of 3). Co-formulation vehicles comprising 22%
10 w/v HP-B-CD or more all resulted in minimal to moderate necrosis and inflammatory cell infiltration (scores of up to 4). Based on these results, co-formulations containing less than 22% HP-B-CD appear suitable for subcutaneous use.

The co-formulation vehicles containing 20% w/v and 22% w/v HP-B-CD and citrate (co-formulation vehicles 15 and 16) resulted in marked necrosis and inflammatory cell
15 infiltration (scores of up to 5). Surprisingly, co-formulation vehicles containing 20% w/v and 22% w/v HP-B-CD, histidine and sorbitol (co-formulation vehicles 11 and 12) were more well tolerated, resulting in moderate necrosis and inflammatory cell infiltration (scores of up to 4).

Co-formulation vehicles comprising m-cresol, phenol and EDTA, and either 15% w/v or 20% w/v HP-B-CD were well tolerated, resulting in mild necrosis and inflammatory cell
20 infiltration (scores of up to 3).

EXAMPLE 18: EFFECT OF DIFFERENT PHARMACEUTICAL PRESERVATIVES ON STAPHYLOCOCCUS AUREUS PRESERVATIVE EFFICACY FOR THE CAGRILINTIDE AND SEMAGLUTIDE CO-FORMULATION

This example shows the effect of different antimicrobial preservatives in the
25 cagrilintide and semaglutide co-formulation on the growth of *Staphylococcus aureus* after 24 hours.

Composition

The composition of co-formulations 45 to 56 is shown in table 36.
30

Table 36 Composition of co-formulations with different types of preservatives

Ingredient	Co-formulation												
	45	46	47	48	49	50	51	52	53	54	55	56	
Cagrilintide drug substance (mg/ml)	3.2												
Semaglutide drug substance (mg/ml)	3.2												
L-Histidine (mM)	6												
Sorbitol (mg/ml)	20												
HP-B-CD KLEPTOSE® (Roquette) (% w/v) (Average MS: 0.62, MS range : 0.58-0.68) ¹	15												
Polysorbate80 (mg/ml)	0.05												
Benzyl alcohol (mg/ml)	9.5	9.5	-	-	-	-	-	-	-	-	-	-	
EDTA disodium, dihydrate (mg/ml)	-	2.0	-	-	-	-	2.0	-	2.0	-	-	-	
m-cresol (mg/ml)	-	-	2.7	2.7	-	-	-	-	-	-	-	-	
Phenol (mg/ml)	-	-	-	2.35	-	-	-	-	-	-	-	-	
Potassium metabisulphite (mg/ml)	-	-	-	-	1.0	-	-	-	-	-	-	-	
Sodium sulphite (mg/ml)	-	-	-	-	-	1.5	1.5	-	-	-	-	-	
Benzalkonium chloride (mg/ml)	-	-	-	-	-	-	-	2.0	2.0	-	-	-	
Propylparaben (mg/ml)	-	-	-	-	-	-	-	-	-	2.0	-	-	
Methylparaben (mg/ml)	-	-	-	-	-	-	-	-	-	-	4.4	-	
Chlorobutanol (mg/ml)	-	-	-	-	-	-	-	-	-	-	-	5.3	
HCl	q.s.												
NaOH	q.s.												
pH	5.7												
Water for injection	To make 1 ml												

¹ MS: molar substitution, corresponds to hydroxypropyls per glucose unit

Preparation

The co-formulations were prepared by making an excipient solution in which the drug substances were dissolved, followed by adjustment to reach final pH and volume. The co-formulations were sterile filtered and filled in 3 ml cartridges.

5 **Methods**

The preservative efficacy test was conducted according to the European Pharmacopoeia (5.1.3) with the exception that it was only tested on the microorganism *Staphylococcus aureus* and the preservative efficacy was only calculated after 24 hours in terms of log reductions in the viable microorganism count compared to the time zero value
10 obtained for the inoculum.

Table 37 Effect of different preservatives on log reductions after 24 hours

Co-formulation	Preservatives	Log reduction after 24 hours <i>S. aureus</i>
45	Benzyl alcohol	-0.4
46	Benzyl alcohol + EDTA	-0.2
47	M-cresol	-0.5
48	M-cresol + phenol	0.4
49	Potassium metabisulfite	4.0
50	Sodium sulfite	4.5
51	Sodium sulfite + EDTA	2.1
52	Benzalkonium	0.1
53	Benzalkonium + EDTA	-0.1
54	Propylparaben	-0.4
55	Methylparaben	-0.3
56	Chlorobutanol	-0.3

15 **Concluding remarks**

The highest reductions in growth of *S. aureus* after 24 hours were obtained in co-formulations 49, 50, and 51 containing either potassium metabisulfite or sodium sulfite. Co-formulation 48 containing m-cresol and phenol showed low log reductions while the remaining co-formulations showed no ability to inhibit the growth of *S. aureus* after 24 hours.
20

EXAMPLE 19: EFFECT OF SODIUM SULFITE AND POTASSIUM METABISULFITE ON THE CHEMICAL STABILITY OF THE CAGRILINTIDE AND SEMAGLUTIDE COFORMUALTION

5 The following example shows the effect of the two preservatives: sodium sulfite and potassium metabisulfite on the chemical stability in terms of chemical purity of cagrilintide and semaglutide in otherwise identical co-formulations.

Composition

The composition of co-formulation 57 to 63 is shown in table 38.

10 **Table 38 Composition of co-formulation 57 to 63 containing sodium sulfite or potassium metabisulfite**

Ingredient	Co-formulation						
	57	58	59	60	61	62	63
Cagrilintide drug substance (mg/ml)	3.2						
Semaglutide drug substance (mg/ml)	3.2						
L-Histidine (mM)	6						
Sorbitol (mg/ml)	20						
HP-B-CD KLEPTOSE® (Roquette) (% w/v) (Average MS : 0.62, MS range : 0.58-0.68) ¹	15						
Polysorbate80 (mg/ml)	0.05						
Sodium sulfite (mg/ml)	-	0.5	1.0	1.5	-	-	-
Potassium metabisulfite (mg/ml)	-	-	-	-	0.33	0.66	1.0
HCl	q.s.						
NaOH	q.s.						
pH	5.7						
Water for injection	To make 1 ml						

¹ MS: molar substitution, corresponds to hydroxypropyls per glucose unit

Preparation

The co-formulations were prepared as described in example 18.

15 **Method**

Samples used to determine the purity of cagrilintide and semaglutide were stored at 37°C for up to 21 days

Purity of cagrilintide was determined using the reversed phase high performance liquid chromatography (RP-HPLC) method described in example 1.

5 Purity of semaglutide was determined using the reversed phase high performance liquid chromatography (RP-HPLC) method described in example 3.

Table 39 Chemical purity (%) of cagrilintide in co-formulations containing sodium sulfite or potassium metabisulfite

Co-formulation	Preservative	Cagrilintide purity (%) as a function of time (days) at 37°C		
		0	14	21
57	No preservatives	92.96%	86.89%	82.68%
58	Sodium sulfite: 0.5 mg/ml	76.16%	68.96%	66.42%
59	Sodium sulfite: 1.0 mg/ml	67.92%	62.56%	59.68%
60	Sodium sulfite: 1.5 mg/ml	64.05%	57.51%	54.80%
61	Potassium metabisulfite: 0.33 mg/ml	79.13%	70.76%	68.39%
62	Potassium metabisulfite: 0.66 mg/ml	71.89%	65.55%	62.19%
63	Potassium metabisulfite: 1.0 mg/ml	67.06%	60.92%	57.95%

10

Table 40 Chemical purity (%) of semaglutide in co-formulations containing either sodium sulfite or potassium metabisulfite

Co-formulation	Preservative	Semaglutide purity (%) as a function of time (days) at 37°C		
		0	14	21
57	No preservatives	97.76%	93.87%	93.08%
58	Sodium sulfite: 0.5 mg/ml	92.56%	74.22%	71.28%
59	Sodium sulfite: 1.0 mg/ml	89.84%	75.22%	72.52%
60	Sodium sulfite: 1.5 mg/ml	89.60%	77.06%	76.02%
61	Potassium metabisulfite: 0.33 mg/ml	93.60%	75.87%	72.17%
62	Potassium metabisulfite: 0.66 mg/ml	91.71%	75.31%	71.39%
63	Potassium metabisulfite: 1.0 mg/ml	89.89%	74.69%	72.49%

Concluding remarks

The results presented in table 39 and table 40 show a rapid decrease in purity of cagrilintide and semaglutide when sodium sulfite or potassium metabisulfite is added to the co-formulation, compared to the co-formulation 57 without preservatives. The loss in purity is more rapid at higher concentrations of preservatives.

5 **EXAMPLE 20: EFFECT AFTER 7 DAYS OF DIFFERENT PHARMACEUTICAL PRESERVATIVES ON THE PRESERVATIVE EFFICACY FOR THE CAGRILINTIDE AND SEMAGLUTIDE CO-FORMULATION**

This example shows the effect of different antimicrobial preservatives in the cagrilintide and semaglutide co-formulation on the growth of the microorganisms

10 *Staphylococcus aureus*, *Candida albicans*, and *Aspergillus brasiliensis* after 7 days.

Composition

The composition of co-formulations 45 to 48 and 52 to 56 is as presented in example 18.

15

Preparation

The co-formulations were prepared as described in example 18.

Methods

20

The preservative efficacy test was conducted according to the United States Pharmacopoeia (chapter 51) with the exception that it was only tested on the microorganisms *Staphylococcus aureus*, *Candida albicans*, and *Aspergillus brasiliensis* and the preservative efficacy was only calculated after 7 days in terms of log reductions in the viable microorganism count compared to the time zero value obtained for the inoculum.

25

Table 41 Effect of different preservatives on log reductions after 7 days

Co-formulation	Preservatives	Log reductions after 7 days		
		S. <i>aureus</i>	C. <i>albicans</i>	A. <i>brasiliensis</i>
45	Benzyl alcohol	0.3	-0.6	0.0
46	Benzyl alcohol + EDTA	0.2	0.6	0.1
47	M-cresol	0.1	<-0.5	0.1
48	M-cresol + phenol	0.9	<-0.5	0.2

52	Benzalkonium	1.8	<-0.5	0.0
53	Benzalkonium + EDTA	0.8	0.0	0.2
54	Propylparaben	0.3	<-0.5	0.2
55	Methylparaben	0.3	<-0.5	0.0
56	Chlorobutanol	0.3	<-0.5	0.0

Concluding remarks

The results presented in table 7 shows that for *S. aureus*, the highest log reductions were obtained in co-formulations 47, 52, and 53 containing either m-cresol and phenol or benzalkonium. For *C. albicans*, no growth was seen in co-formulations 46 and 53 containing EDTA. For *A. brasiliensis* no difference between the tested co-formulations was seen.

EXAMPLE 21: EFFECT AFTER 7 DAYS OF DIFFERENT CONCENTRATIONS OF BENZALKONIUM CHLORIDE AND EDTA ON THE PRESERVATIVE EFFICACY FOR THE CAGRILINTIDE AND SEMAGLTUDIE CO-FORMULATION

This example shows the effect of cagrilintide and semaglutide co-formulations containing different concentrations of benzalkonium chloride and EDTA disodium, dihydrate on the growth of *Staphylococcus aureus*, *Candida albicans*, and *Aspergillus brasiliensis* after 7 days.

Composition

The composition of co-formulations 64 to 67 is shown in table 42.

Table 42 Composition of co-formulations containing different concentrations of with benzalkonium and EDTA

Ingredient	Co-formulation			
	64	65	66	67
Cagrilintide drug substance (mg/ml)	3.2			
Semaglutide drug substance (mg/ml)	3.2			
L-Histidine (mM)	6			
Sorbitol (mg/ml)	20			
HP-B-CD	15			

KLEPTOSE® (Roquette) (% w/v) (Average MS : 0.62, MS range : 0.58-0.68) ¹				
Polysorbate80 (mg/ml)	0.05			
Benzalkonium chloride (mg/ml)	1.0	2.0	2.0	2.0
EDTA disodium, dihydrate (mg/ml)	1.0	0.5	1.0	2.0
HCl	q.s.			
NaOH	q.s.			
pH	5.8			
Water for injection	To make 1 ml			

¹ MS: molar substitution, corresponds to hydroxypropyls per glucose unit

Preparation

The co-formulations were prepared as described in example 18.

5

Methods

The preservative efficacy of the co-formulations was tested on the microorganisms *Staphylococcus aureus*, *Candida albicans*, and *Aspergillus brasiliensis* and the preservative efficacy was calculated in terms of log reductions in the viable microorganism count after 7 days compared to the time zero value obtained for the inoculum.

10

Table 43 Effect of different preservatives on log reductions after 7 days

Co- formulation	Benzalkonium chloride / EDTA disodium, dihydrate concentrations	Log reductions after 7 days		
		S. <i>aureus</i>	C. <i>albicans</i>	A. <i>brasiliensis</i>
64	Benzalkonium chloride: 1.0 mg/ml EDTA disodium, dihydrate : 1.0 mg/ml	1.0	-0.1	0.1
65	Benzalkonium chloride: 2.0 mg/ml EDTA disodium, dihydrate: 0.5 mg/ml	1.1	-0.3	0.1
66	Benzalkonium chloride: 2.0 mg/ml EDTA disodium, dihydrate: 1.0 mg/ml	1.5	0.0	0.0
67	Benzalkonium chloride: 2.0 mg/ml EDTA disodium, dihydrate: 2.0 mg/ml	1.5	0.2	0.0

Concluding remarks

The results presented in table 43 show that higher log reductions for *S. aureus* were obtained with higher concentrations of benzalkonium. Increasing concentrations of EDTA resulted in higher log reductions for *C. albicans*, while no difference was observed between the different co-formulations for *A. brasiliensis*.

5 **EXAMPLE 22: EFFECT OF DIFFERENT CONCENTRATIONS OF BENZALKONIUM AND EDTA ON THE CHEMICAL STABILITY OF THE CAGRILINTIDE AND SEMAGLUTIDE CO-FORMULATION**

The following example shows the effect of different concentrations of benzalkonium and EDTA on the chemical stability in terms of chemical purity of cagrilintide and
10 semaglutide.

Composition

The composition of co-formulations 64 to 68 is shown in table 44.

15 **Table 44 Composition of co-formulation 64 to 68 with benzalkonium and EDTA**

Ingredient	Co-formulation				
	64	65	66	67	68
Cagrilintide drug substance (mg/ml)	3.2				
Semaglutide drug substance (mg/ml)	3.2				
L-Histidine (mM)	6				
Sorbitol (mg/ml)	20				
HP-B-CD KLEPTOSE® (Roquette) (% w/v) (Average MS : 0.62, MS range : 0.58-0.68) ¹	15				
Polysorbate80 (mg/ml)	0.05				
Benzalkonium chloride (mg/ml)	1.0	2.0	2.0	2.0	-
EDTA disodium, dihydrate (mg/ml)	1.0	0.5	1.0	2.0	-
HCl	q.s.				
NaOH	q.s.				
pH	5.8				
Water for injection	To make 1 ml				

¹ MS: molar substitution, corresponds to hydroxypropyls per glucose unit

Preparation

The co-formulations were prepared as described in example 18.

Methods

5 Samples used to determine the purity of cagrilintide and semaglutide were stored at 37°C for 6 weeks.

Purity of cagrilintide and semaglutide was determined using the reversed phase high performance liquid chromatography (RP-HPLC) method described in example 13.

10 **Table 45 Chemical purity (%) of cagrilintide in co-formulations with different concentrations of benzalkonium and EDTA**

Co-formulation	Benzalkonium chloride / EDTA disodium, dihydrate concentrations	Cagrilintide purity (%) after 6 weeks at 37°C
64	Benzalkonium chloride: 1.0 mg/ml EDTA disodium, dihydrate : 1.0 mg/ml	59.4%
65	Benzalkonium chloride: 2.0 mg/ml EDTA disodium, dihydrate: 0.5 mg/ml	35.5%
66	Benzalkonium chloride: 2.0 mg/ml EDTA disodium, dihydrate: 1.0 mg/ml	35.3%
67	Benzalkonium chloride: 2.0 mg/ml EDTA disodium, dihydrate: 2.0 mg/ml	35.6%
68	No preservatives	86.0%

15 **Table 46 Chemical purity (%) of semaglutide in co-formulations with different concentrations of benzalkonium and EDTA**

Co-formulation	Benzalkonium chloride / EDTA disodium, dihydrate concentrations	Semaglutide purity (%) after 6 weeks at 37°C
64	Benzalkonium chloride: 1.0 mg/ml EDTA disodium, dihydrate : 1.0 mg/ml	94.8%
65	Benzalkonium chloride: 2.0 mg/ml EDTA disodium, dihydrate: 0.5 mg/ml	94.6%

66	Benzalkonium chloride: 2.0 mg/ml EDTA disodium, dihydrate: 1.0 mg/ml	94.5%
67	Benzalkonium chloride: 2.0 mg/ml EDTA disodium, dihydrate: 2.0 mg/ml	94.5%
68	No preservatives	94.4%

Concluding remarks

5 The results presented in table 45 show that the chemical purity of cagrilintide in co-formulations 64 to 67 containing different concentrations of benzalkonium and EDTA were significantly lower compared to co-formulation 68 without preservatives. The results in table 46 show a little positive effect on the chemical purity of semaglutide.

EXAMPLE 23: EFFECT AFTER 28 DAYS OF DIFFERENT TONICITY AGENTS ON THE PRESERVATIVE EFFICACY FOR THE PRESERVED CAGRILINTIDE AND SEMAGLTUDIE CO-FORMULATION

10 This example shows the effect of two different tonicity agents: sorbitol or propylene glycol together with m-cresol, phenol and EDTA, on preservative efficacy of the cagrilintide and semaglutide co-formulation when tested in a full preservative efficacy test (Ph. Eur. 5.1.3, USP <51>).

15 **Composition**

 The composition of co-formulation 69 and 70 is shown in table 47.

Table 47 Composition of co-formulation containing m-cresol, phenol, and EDTA and either sorbitol or propylene glycol

Ingredient	Co-formulation	
	69	70
Cagrilintide drug substance (mg/ml)	3.2	
Semaglutide drug substance (mg/ml)	3.2	
L-Histidine (mM)	6	
Sorbitol (mg/ml)	20	-
Propylene glycol (mg/ml)	-	8.3
HP-B-CD KLEPTOSE® (Roquette) (% w/v)	15	

(Average MS : 0.62, MS range : 0.58-0.68) ¹	
Polysorbate80 (mg/ml)	0.05
m-cresol (mg/ml)	2.7
Phenol (mg/ml)	2.35
EDTA disodium, dihydrate (mg/ml)	2.0
HCl	q.s.
NaOH	q.s.
pH	5.8
Water for injection	To make 1 ml

¹ MS: molar substitution, corresponds to hydroxypropyls per glucose unit

Preparation

The co-formulations were prepared as described in example 18.

5

Methods

The preservative efficacy of the co-formulations was tested in a full preservative efficacy test according to USP (<51>) and Ph. Eur. (5.1.3.) on the microorganisms:

10 *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Candida albicans* and *Aspergillus brasiliensis*. The preservative efficacy was calculated in terms of log reductions in the viable microorganism count compared to the time zero value obtained for the inoculum.

Table 48 Effect of m-cresol, phenol and EDTA with different tonicity agents on preservative efficacy

Co-formulation	Tonicity agent	Microorganism	Log reductions		
			7 days	14 days	28 days
69	Sorbitol	<i>S. aureus</i>	1.3	4.7	5.6
70	Propylene glycol		1.1	4.6	5.6
69	Sorbitol	<i>P. aeruginosa</i>	5.5	5.5	5.5
70	Propylene glycol		5.5	5.5	5.5
69	Sorbitol	<i>E. coli</i>	1.4	2.0	3.3
70	Propylene glycol		1.5	2.2	3.5
69	Sorbitol	<i>C. albicans</i>	0.5	0.6	2.0
70	Propylene glycol		0.3	0.7	2.2
69	Sorbitol	<i>A. brasiliensis</i>	0.6	2.0	3.3

70	Propylene glycol		0.7	2.2	2.7
----	------------------	--	-----	-----	-----

Concluding remarks

Overall, no difference in the preservative efficacy was seen between co-formulations 69 and 70 containing either sorbitol or propylene glycol as tonicity agent.

5 **EXAMPLE 24: EFFECT OF DIFFERENT TONICITY AGENTS ON THE CHEMICAL STABILITY OF PRESERVED CAGRILINTIDE AND SEMAGLUTIDE CO-FORMULATION**

This example shows the effect of m-cresol, phenol, EDTA with two different tonicity agents: sorbitol or propylene glycol on the chemical stability of the cagrilintide and semaglutide co-formulation measured as the purity of cagrilintide and semaglutide.

10

Composition

The composition of co-formulation 69 and 70 is shown example 23.

Preparation

15

The co-formulation were prepared as described in example 18.

Methods

Samples used to determine the purity of cagrilintide and semaglutide were stored at 37°C for 4 weeks.

20

Purity of cagrilintide and semaglutide was determined using the reversed phase high performance liquid chromatography (RP-HPLC) method described in example 13.

25 **Table 49 Chemical purity (%) of cagrilintide in co-formulations with either sorbitol or propylene glycol as tonicity agent**

Co-formulation	Tonicity agent	Cagrilintide purity (%) after 4 weeks at 37°C
69	Sorbitol	80.62%
70	Propylene glycol	80.23%

Table 50 Chemical purity (%) of semaglutide in co-formulations with either sorbitol or propylene glycol as tonicity agent

Co-formulation	Tonicity agent	Semaglutide purity (%) after 4 weeks at 37°C
69	Sorbitol	93.86%
70	Propylene glycol	94.06%

Concluding remarks

The results presented in table 49 and table 50 show that no difference was observed for the chemical purity of cagrilintide and semaglutide for co-formulations preserved with m-cresol, phenol and EDTA containing either sorbitol or propylene glycol as tonicity agent.

EXAMPLE 25: EFFECT OF POLYSORBATE 80 ON THE PHYSICAL STABILITY OF THE CAGRILINTIDE AND SEMAGLTUDIE CO-FORMULATION PRESERVED WITH M-CRESOL, PHENOL AND EDTA

This example shows the effect of polysorbate 80 on the physical stability in terms of sub-visible particle count in otherwise identical cagrilintide and semaglutide co-formulations preserved with m-cresol, phenol and EDTA.

Composition

The composition of co-formulations 71 to 72 is shown in table 51.

Table 51 Composition of co-formulations with and without polysorbate 80

Ingredient	Co-formulation	
	71	72
Cagrilintide drug substance (mg/ml)	3.2	
Semaglutide drug substance (mg/ml)	3.2	
L-Histidine (mM)	6	
Sorbitol (mg/ml)	20	
HP-B-CD KLEPTOSE® (Roquette) (% w/v) (Average MS : 0.62, MS range : 0.58-0.68) ¹	15	
Polysorbate80 (mg/ml)	-	0.05

m-cresol (mg/ml)	2.48
Phenol (mg/ml)	2.16
EDTA disodium, dihydrate (mg/ml)	1.84
HCl	q.s.
NaOH	q.s.
pH	5.8
Water for injection	To make 1 ml

¹ MS: molar substitution, corresponds to hydroxypropyls per glucose unit

Preparation

The co-formulation were prepared as described in example 18.

5

Methods

Samples used to determine the number of sub-visible particles were stored at stressed conditions, defined as:

- Duration: 28 days
- 10 - Temperature: 30°C ± 2°C
- Stress condition: During storage, samples were inverted 360° to simulate patient use out of refrigerated storage. The rotations were performed 20 times three days every week, and 40 times two days every week.

The number of sub-visible particles was quantified as described in example 5.

15

Table 52 Levels of sub-visible particles in co-formulations

Co- formulation	Surfactant	Number of sub-visible particles as a function of time (days)			
		0	14	21	28
Particle size >5µm					
71	-	4638	3678	1665	3103
72	Polysorbate 80	32	171	185	962
Particle size >10µm					
71	-	472	1097	434	1948
72	Polysorbate 80	6	47	52	285

Particle size >25µm					
71	-	0	28	18	110
72	Polysorbate 80	0	6	10	25

Results for number of sub-visible particles are the mean of 3 replicates and has been rounded to nearest integer value

Concluding remarks

5 The data in table 52 show that the number of sub-visible particles seen in co-formulation 71 without polysorbate 80 was greater than the number of sub-visible particles seen in co-formulation 72 containing polysorbate 80. That is, the co-formulation comprising polysorbate 80 was the most physically stable.

EXAMPLE 26: EFFECT OF THE MOLAR SUBSTITUTION OF HP-B-CD ON THE PRESERVATIVE EFFICACY OF THE CAGRILINTIDE AND SEMAGLUTIDE CO-FORMULATION PRESERVED WITH M-CRESOL, PHENOL AND EDTA

10 This example shows the effect of HP-B-CD molar substitution on preservative efficacy of the cagrilintide and semaglutide co-formulation preserved with m-cresol, phenol, EDTA when tested in a full preservative efficacy test (Ph. Eur. 5.1.3, USP <51>).

Composition

15 The composition of co-formulations 73 to 74 is shown in table 53.

Table 53 Composition of m-cresol, phenol and EDTA preserved co-formulation containing HP-B-CD varying hydroxypropyl molar substitution degree

Ingredient	Co-formulation	
	73	74
Cagrilintide drug substance (mg/ml)	3.2	
Semaglutide drug substance (mg/ml)	3.2	
L-Histidine (mM)	6	
Sorbitol (mg/ml)	20	
HP-B-CD KLEPTOSE® (Roquette) (% w/v) (Average MS : 0.62, MS range : 0.58-0.68) ¹	15	-

HP-B-CD KLEPTOSE® (Roquette) (% w/v) (Average MS: 0.92, MS range: 0.81-0.99) ¹	-	15
Polysorbate80 (mg/ml)	0.05	
m-cresol (mg/ml)	2.48	
Phenol (mg/ml)	2.16	
EDTA disodium, dihydrate (mg/ml)	1.84	
HCl	q.s.	
NaOH	q.s.	
pH	5.8	
Water for injection	To make 1 ml	

¹ MS: molar substitution, corresponds to hydroxypropyls per glucose unit

Preparation

The co-formulations were prepared as described in example 18.

5

Methods

The preservative efficacy of the co-formulations was tested in a full preservative efficacy test according to USP (<51>) and Ph. Eur. (5.1.3.) on the microorganisms *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Candida albicans* and *Aspergillus brasiliensis*. The preservative efficacy was calculated in terms of log reductions in the viable microorganism count compared to the time zero value obtained for the inoculum.

10

Table 54 Effect of molar substitution of HP-B-CD on preservative efficacy of co-formulations preserved with m-cresol, phenol and EDTA

Co-formulation	HP-B-CD molar substitution ¹	Microorganism	Log reductions		
			7 days	14 days	28 days
73	0.62	<i>S. aureus</i>	2.0	5.2	5.2
74	0.92		4.6	5.2	5.2
73	0.62	<i>P. aeruginosa</i>	5.1	5.1	5.1
74	0.92		5.1	5.1	5.1
73	0.62	<i>E. coli</i>	1.9	3.2	4.3

74	0.92		3.4	4.3	4.3
73	0.62	<i>C. albicans</i>	0.5	0.8	1.5
74	0.92		0.2	0.4	1.1
73	0.62	<i>A. brasiliensis</i>	0.2	1.6	3.1
74	0.92		0.3	1.8	3.1

¹ MS: molar substitution, corresponds to hydroxypropyls per glucose unit

Concluding remarks

The results shown in table 54 in form of log reductions for *S. aureus*, *E. coli* and *A. brasiliensis* were increased in co-formulation 74 (Average MS: 0.92) compared to co-formulation 73 (Average MS: 0.62), while greater log reductions for *C. albicans* were seen for co-formulation 73 (Average MS: 0.62). No difference between the two co-formulations was seen for *P. aeruginosa*.

10 **EXAMPLE 27: EFFECT OF THE MOLAR SUBSTITUTION OF HP-B-CD ON THE PHYSICAL STABILITY OF THE PRESERVED CAGRILINTIDE AND SEMAGLTUDIE CO-FORMULATION**

This example shows the effect of HP-B-CD molar substitution degree on the physical stability in terms of sub-visible particle count in otherwise identical preserved
15 cagrilintide and semaglutide co-formulations.

Composition

The composition of co-formulations 73 to 74 is as shown in example 26.

20 **Preparation**

The co-formulations were prepared as described in example 18.

Methods

Samples used to determine the number of sub-visible particles were stored at
25 stressed conditions, defined as:

- Duration: 35 days
- Temperature: 30°C ± 2°C

- Stress condition: During storage, samples were inverted 360° to simulate patient use out of refrigerated storage. The rotations were performed 20 times three days every week, and 40 times two days every week.
The number of sub-visible particles was quantified as described in example 5.

5

Table 55 **Levels of sub-visible particles in co-formulations**

Co- formulation	HP-B-CD molar substitution ¹	Number of sub-visible particles as a function of time (days)	
		0	35
Particle size >5µm			
73	0.62	115	3396
74	0.92	244	105
Particle size >10µm			
73	0.62	26	936
74	0.92	19	11
Particle size >25µm			
73	0.62	0	66
74	0.92	0	0

Results for number of sub-visible particles are the mean of 3 replicates and has been rounded to nearest integer value

10 **Concluding remarks**

The results presented in table 55 show that the overall level of sub-visible particles after 35 days was lower in co-formulation 74 (Average MS: 0.92) compared to co-formulation 73 (Average MS: 0.62). In this otherwise identical co-formulation of semaglutide and cagrilintide, co-formulation 74 comprising HP-B-CD (Average MS: 0.92) was more physically

15 stable than co-formulation 73 comprising HP-B-CD (Average MS: 0.62).

EXAMPLE 28: EFFECT OF CONCENTRATION OF M-CRESOL, EDTA AND HP-B-CD ON THE PRESERVATIVE EFFICACY OF THE PRESERVED CAGRILINTIDE AND SEMAGLUTIDE CO-FORMULATION

5 This example shows the effect of different concentration of m-cresol, EDTA and HP-B-CD on preservative efficacy of the preserved cagrilintide and semaglutide coformulation when tested for preservative efficacy.

Composition

The composition of coformulation 75 to 78 is shown in table 56.

10

Table 56 Composition of co-formulations with different concentration of m-cresol, EDTA and HP-B-CD

Ingredient	Co-formulation			
	75	76	77	78
Cagrilintide drug substance (mg/ml)	3.2			
Semaglutide drug substance (mg/ml)	3.2			
Histidine (mM)	6			
Sorbitol (mg/ml)	20			
HP-B-CD KLEPTOSE® (Roquette) (% w/v) (Average MS: 0.92, MS range: 0.81-0.99) ¹	15	18		
Polysorbate80 (mg/ml)	0.05			
m-cresol (mg/ml)	2.70	2.70	2.16	2.70
EDTA disodium, dihydrate (mg/ml)	2.0	2.0	2.0	3.0
Phenol (mg/ml)	2.35			
HCl	q.s.			
NaOH	q.s.			
pH	6.0			
Water for injection	To make 1 ml			

¹ MS: molar substitution, corresponds to hydroxypropyls per glucose unit

15

Preparation

The coformulation were prepared as described in example 18.

Methods

The preservative efficacy of the co-formulations was tested on the microorganisms *Staphylococcus aureus*, *Candida albicans*, and *Aspergillus brasiliensis* and the preservative efficacy was calculated in terms of log reductions in the viable microorganism count after 7 days compared to the time zero value obtained for the inoculum.

5 The method for testing preservative efficacy of 4 co-formulations was conducted according to the United States Pharmacopoeia (chapter 51), and Ph. Eur. (5.1.3.) with the exception that the preservative efficacy was only tested on the microorganism *Candida albicans* and only calculated after 7 days in terms of log reductions in the viable microorganism count compared to the time zero value obtained for the inoculum.

10

Table 57 Effect of different concentrations of m-cresol, EDTA and HP-B-CD on preservative efficacy for *C. albicans*

Co-formulation	Log reductions for <i>C. albicans</i> after 7 days
75	0.2
76	0.2
77	0.2
78	0.6

Concluding remarks

15 The results presented in table 57 showed no difference between co-formulation 75, 76, and 77 comprising difference concentrations of HP-B-CD and m-cresol. For co-formulation 78 containing higher concentrations of EDTA, higher log reductions for *C. albicans* was seen.

20 **EXAMPLE 29: EFFECT OF THE MOLAR SUBSTITUTION OF HP-B-CD ON THE PHYSICAL STABILITY OF THE PRESERVED CAGRILINTIDE AND SEMAGLTUDIE CO-FORMULATION**

 This example shows the effect of HP-B-CD molar substitution degree on the physical stability in terms of sub-visible particle count in otherwise identical preserved cagrilintide and semaglutide co-formulations.

25

Composition

 The composition of co-formulations 79 to 84 is as shown in table 58.

Table 58 **Composition of co-formulations comprising HP-B-CD with different average molar substitution degrees**

Ingredient	Co-formulation					
	79	80	81	82	83	84
Cagrilintide drug substance (mg/ml)	3.2					
Semaglutide drug substance (mg/ml)	3.2					
Histidine (mM)	6					
Sorbitol (mg/ml)	20					
HP-B-CD KLEPTOSE® (Roquette) (% w/v) (Average MS: 0.62, MS range: 0.58-0.68) ¹	15	-	-	-	-	-
HP-B-CD (CycloLab) (% w/v) (Average MS: 0.67, MS range: 0.6-0.9) ¹	-	15	-	-	-	-
HP-B-CD Cavitron® (Ashland) (% w/v) (Average MS: 0.68, MS range: 0.58-0.72) ¹	-	-	15	-	-	-
HP-B-CD Trappsol® (CTD. Inc.) (% w/v) (Average MS: 0.84, MS range: 0.8-1.0) ¹	-	-	-	15	-	-
HP-B-CD KLEPTOSE® (Roquette) (% w/v) (Average MS: 0.92, MS range: 0.81-0.99) ¹	-	-	-	-	15	-
HP-B-CD Cavitron® (Ashland) (% w/v) (Average MS: 1.08, MS range: 0.86-1.14) ¹	-	-	-	-	-	15
Polysorbate80 (mg/ml)	0.05					
m-cresol (mg/ml)	3.78					
EDTA disodium, dihydrate (mg/ml)	2.0					
Phenol (mg/ml)	2.35					
HCl	q.s.					
NaOH	q.s.					
pH	6.0					

Water for injection	To make 1 ml
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¹ MS: molar substitution, corresponds to hydroxypropyls per glucose unit

Preparation

5 The co-formulations were prepared by making an excipient solution containing HP-B-CD, histidine, and sorbitol in which the drug substances are added. Hereafter, polysorbate80 and preservatives are added followed by adjustment to reach final pH and volume. The co-formulations were sterile filtered and filled in 3 ml cartridges.

Methods

10 Samples used to determine the number of sub-visible particles were stored at stressed conditions, defined as:

- Duration: 28 days
- Temperature: 30°C ± 2°C
- Stress condition: During storage, samples were inverted 360° to simulate patient

15 use out of refrigerated storage. The rotations were performed 20 times three days every week, and 40 times two days every week.

 The number of sub-visible particles was quantified as described in example 5.

Table 59 Levels of sub-visible particles in co-formulations

Co- formulation	HP-B-CD molar substitution ¹	Number of sub-visible particles after 28 days
Particle size >10µm		
79	0.62	77
80	0.67	69
81	0.68	69
82	0.84	90
83	0.92	14
84	1.08	26676
Particle size >25µm		
79	0.62	3
80	0.67	3
81	0.68	3
82	0.84	3
83	0.92	0
84	1.08	5040

Results for number of sub-visible particles are the mean of 3 replicates and has been rounded to nearest integer value

¹ MS: molar substitution, corresponds to hydroxypropyls per glucose unit

5

Concluding remarks

The results presented in table 59 show that in otherwise identical co-formulations of semaglutide and cagrilintide, the number of sub-visible particles counted, after 28 days, in co-formulation 84 (comprising HP-B-CD with an average molar substitution degree of 1.08) was unacceptably high compared to the other co-formulations with a lower average molar substitution degree. The co-formulations comprising a HP-B-CD with an average molar substitution degree of 0.62, 0.67, 0.68, 0.84 and 0.92 were physically stable.

10

**EXAMPLE 30: EFFECT OF THE MOLAR SUBSTITUTION OF HP-B-CD ON THE
CHEMICAL STABILITY OF THE PRESERVED CAGRILINTIDE AND SEMAGLTUDIE CO-
FORMULATION**

5 This example shows the effect of HP-B-CD molar substitution degree on the
chemical stability measured as the purity of semaglutide in otherwise identical preserved
cagrilintide and semaglutide co-formulations.

Composition

10 The composition of co-formulations 79 to 84 is as shown in example 29.

Preparation

The co-formulations were prepared as described in example 29.

Methods

15 Samples used to determine the purity of cagrilintide and semaglutide were stored at
37°C for 4 weeks.

Purity loss of semaglutide was determined using the reversed phase high
performance liquid chromatography (RP-HPLC) method described in example 13.

20 **Table 60 Chemical purity (%) of semaglutide in co-formulations containing
HP-B-CD with difference molar substitution**

Co-formulation	HP-B-CD molar substitution ¹	Semaglutide purity loss (%) after 4 weeks at 37°C
79	0.62	3.3%
80	0.67	2.8%
81	0.68	2.8%
82	0.84	2.6%
83	0.92	3.1%
84	1.08	8.1%

¹ MS: molar substitution, corresponds to hydroxypropyls per glucose unit

Concluding remarks

25 The results presented in table 60 show that the chemical purity of semaglutide was
affected by the average molar substitution degree of HP-B-CD. Loss of semaglutide chemical
purity was greatest in co-formulation 84 (Average MS: 1.08). The co-formulations comprising

a HP-B-CD with an average molar substitution degree of 0.62, 0.67, 0.68, 0.84 or 0.92 were all chemically stable.

EXAMPLE 31: EFFECT OF THE PH ON THE PHYSICAL STABILITY OF THE PRESERVED CAGRILINTIDE AND SEMAGLUTIDE CO-FORMULATION

5 This example shows the effect of pH on the physical stability in terms of the propensity of cagrilintide and/or semaglutide to aggregate and form peptide fibrils in otherwise identical preserved cagrilintide and semaglutide co-formulations.

Composition

10 The composition of co-formulations 85 to 89 is as shown in table 61.

Table 61 Composition of co-formulations with different pH

Ingredient	Co-formulation				
	85	86	87	88	89
Cagrilintide drug substance (mg/ml)	3.2				
Semaglutide drug substance (mg/ml)	3.2				
Histidine (mM)	6				
Sorbitol (mg/ml)	20				
HP-B-CD KLEPTOSE® (Roquette) (% w/v) (Average MS: 0.92, MS range: 0.81-0.99) ¹	15				
Polysorbate80 (mg/ml)	0.05				
m-cresol (mg/ml)	3.78				
EDTA disodium, dihydrate (mg/ml)	2.0				
Phenol (mg/ml)	2.35				
HCl	q.s.				
NaOH	q.s.				
pH	5.6	5.8	6.0	6.2	6.4
Water for injection	To make 1 ml				

¹ MS: molar substitution, corresponds to hydroxypropyls per glucose unit

Preparation

The co-formulations were prepared as described in example 29.

Methods

The propensity of cagrilintide and/or semaglutide to aggregate and form peptide fibrils, parameters used to quantify physical stability was quantified as described in example 2.

5

Table 62 Physical stability for cagrilintide and semaglutide co-formulations with varying pH

Co-formulation	pH	Lag time until fibrillation
85	5.6	2.93 hours ¹
86	5.8	6.76 hours ¹
87	6.0	9.50 hours ¹
88	6.2	6.92 hours ¹
89	6.4	5.75 hours ¹

¹ Result is the mean of 6 replicates

10 Concluding remarks

The co-formulations in table 62 were subjected to stress-inducing conditions and the propensity of cagrilintide and/or semaglutide to form peptide fibrils was quantified by measuring the time from the initiation of the assay until fibrillation occurs ("lag time"). The longer the lag time, the more stable the co-formulation. The results show that the lag time until fibrillation is longest in, and thereby the most stable co-formulation is, co-formulation 87 having a pH of 6. Co-formulation 86 (pH 5.8) and co-formulation 88 (pH 6.2) were found to be comparably stable. Co-formulation 85 (pH 5.6) had the shortest lag time until fibrillation, meaning that it was the least unstable, with regard to physical stability, of those tested.

15

20 EXAMPLE 32: EFFECT OF THE CONCENTRATION OF HP-B-CD ON THE PHYSICAL STABILITY OF THE PRESERVED CAGRILINTIDE AND SEMAGLTUDIE CO-FORMULATION

This example shows the effect of HP-B-CD concentration on the physical stability in terms of sub-visible particle count in otherwise identical preserved cagrilintide and semaglutide co-formulations.

25

Composition

The composition of co-formulations 90 to 94 is as shown in table 64.

Table 64 **Composition of co-formulations with different concentrations of HP-B-CD**

Ingredient	Co-formulation				
	90	91	92	93	94
Cagrilintide drug substance (mg/ml)	3.2				
Semaglutide drug substance (mg/ml)	3.2				
Histidine (mM)	6				
Sorbitol (mg/ml)	20				
HP-B-CD KLEPTOSE® (Roquette) (% w/v) (Average MS: 0.92, MS range: 0.81-0.99) ¹	0	5	10	12.5	15
Polysorbate80 (mg/ml)	0.05				
m-cresol (mg/ml)	3.78				
EDTA disodium, dihydrate (mg/ml)	2.0				
Phenol (mg/ml)	2.35				
HCl	q.s.				
NaOH	q.s.				
pH	6.0				
Water for injection	To make 1 ml				

¹ MS: molar substitution, corresponds to hydroxypropyls per glucose unit

5

Preparation

The co-formulations were prepared as described in example 29.

Co-formulation	HP-B-CD (MS: 0.92) ¹	Lag time until fibrillation
90 ²	0% w/v	-
91 ²	5% w/v	-
92	10% w/v	2.92 hours ³
93	12.5% w/v	11.28 hours ³
94	15% w/v	16.65 hours ³

¹ MS: molar substitution, corresponds to hydroxypropyls per glucose unit

² Co-formulation 90 (0% w/v) and 91 (5% w/v) could not be manufactured because the solution was unclear and precipitation was observed results in no data was obtained for the affected batches.

³ Result is the mean of 6 replicates

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Concluding remarks

The results presented in Table 65 show that cagrilintide and semaglutide co-formulations containing less than 10% w/v HP-B-CD (Average MS: 0.92) could not be manufactured due to precipitation. All co-formulations containing more than 10% w/v were deemed physically stable. The longest lag time until fibrillation occurred in co-formulation 94 comprising 15% w/v HP-B-CD, while the lag time until fibrillation in co-formulation 93 comprising 12.5% w/v was longer than for co-formulation 92 comprising 10% w/v. That is, the cagrilintide and semaglutide co-formulations containing a higher content of HP-B-CD (Average MS: 0.92) were more physically stable.

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While certain features of the invention have been illustrated and described herein, many modifications, substitutions, changes, and equivalents will now occur to those of ordinary skill in the art. It is, therefore, to be understood that the appended claims are intended to cover all such modifications and changes as fall within the true spirit of the invention.

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CLAIMS

1. A pharmaceutical formulation comprising an amylin receptor agonist, a GLP-1 receptor agonist, a cyclodextrin of the hydroxypropyl-substituted alpha and/or beta type and at least one preservative; wherein said GLP-1 receptor agonist has a theoretically calculated isoelectric point which is equal to or less than about 4.5, such as about 4.0-4.5; and wherein said cyclodextrin comprises 0.58-1.0 hydroxypropyls per glucose unit.
2. The pharmaceutical formulation according to claim 1, wherein the amylin receptor agonist has a theoretically calculated isoelectric point (pI) of about 7.6-9.4 or 8-9.
3. A pharmaceutical formulation comprising cagrilintide, semaglutide, a cyclodextrin of the hydroxypropyl-substituted alpha and/or beta type, said cyclodextrin comprising 0.58-1.0 hydroxypropyls per glucose unit, and at least one preservative.
4. The pharmaceutical formulation according to any one of claims 1-3, wherein said cyclodextrin is of the hydroxypropyl-substituted beta type.
5. The pharmaceutical formulation according to any one of claims 1-4, wherein said cyclodextrin comprises an average molar substitution (MS) of about 0.92 hydroxypropyls per glucose unit.
6. The pharmaceutical formulation according to any one of claims 1-5, further comprising a buffer having at least one pKa of about 5.0-7.0, such as citrate and/or histidine.
7. The pharmaceutical formulation according to any one of claims 1-6, further comprising a tonicity agent, with the proviso that the tonicity agent is not sodium chloride.
8. The pharmaceutical formulation according to any one of claims 1-7, further comprising a surfactant, such as polysorbate 20 and/or polysorbate 80; preferably, polysorbate 80.

9. The pharmaceutical formulation according to any one of claims 1-3, which is a liquid formulation.
10. The pharmaceutical formulation according to claim 9, wherein the pH is 5.6-6.4, such as 5.7-6.4, such as about 5.7, about 5.8, about 5.9, about 6.0, about 6.1, about 6.2, about 6.3 or about 6.4; preferably, about 5.8-6.2.
11. The pharmaceutical formulation according to any one of claims 9-10, comprising at least 10% w/v and less than 22% w/v cyclodextrin, such as 10-20% w/v cyclodextrin, such as about 15% w/v cyclodextrin.
12. The pharmaceutical formulation according to any one of claims 1-11, wherein said at least one preservative is phenol and/or m-cresol and/or EDTA.
13. The pharmaceutical formulation according to any one of claims 9-12, comprising m-cresol in a concentration of 9-40 mM and/or phenol in a concentration of 18-65 mM and/or EDTA in a concentration of 0.5-5.0 mg/ml.
14. A pharmaceutical formulation comprising:
- 0.25-22 mg/ml cagrilintide,
 - 0.25-22 mg/ml semaglutide,
 - more than 10% w/v and less than 22% w/v, such as 10-20% w/v cyclodextrin of the hydroxypropyl-substituted alpha and/or beta type, wherein said cyclodextrin comprises 0.58-1.0 hydroxypropyls per glucose unit,
 - about 18-65 mM phenol and/or 9-40 mM m-cresol and/or 0.5-5.0 mg/ml EDTA,
 - about 3-30 mM of a buffer having at least one pKa value of about 5.0-7.0, such as histidine or citrate,
 - about 5-35 mg/ml sorbitol,
 - up to 1.0 mg/ml polysorbate 20 and/or 80; such as 0.01-0.1 mg/ml, such as 0.05 mg/ml; or more than 0.1 and less than 0.2 mg/ml polysorbate 20 and/or 80,
 - about 75-90% w/w water and
- having a pH of about 5.6-6.4, such as about 5.8-6.2.
15. The pharmaceutical formulation according to any one of claims 1-14 for use as a medicament.

16. A kit comprising the pharmaceutical composition as defined in any one of claims 1-15 and instructions for use.