PROCESS FOR THE PREPARATION OF A PEPTIDE POWDER FORM

The invention refers to the preparation of a peptide powder form, particularly to a freely flowable homogenous powder form of a GLP-1 peptide drug.

Suitable peptide drugs are analogues of human glucagon-like peptide-1 (GLP-1), particularly the GLP-1 analogue with the amino acid sequence according to SEQ ID No. 1:

His-Aib-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Lys-Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys-Aib-Arg-NH_2,

wherein 26 of these amino acids are in the natural L configuration while four are not chiral. Aib means α-aminoisobutyric acid. This peptide is also named ($Aib^{8,35}$)GLP-1(7-36)NH_2 and its pharmaceutical use and preparation by solid phase peptide synthesis (SPPS) are described in the PCT Publication WO 2000/34331.

The synthesis of GLP-1 analogues can also follow a hybrid approach encompassing both solid phase peptide synthesis (SPPS) and fragment couplings in solution. For example the PCT Publication WO 2007/147816 describes the preparation of ($Aib^{8,35}$) GLP-1(7-36)NH_2 by preparing three fragments and coupling these fragments in solution.

The individual synthetic steps usually are highly selective, however, at the end of a multi-step chemical synthesis the product is typically not pure enough to be used as a drug. The crude product can therefore be subjected to reverse phase high performance liquid chromatography (RP-HPLC), to further purify the peptide and to achieve purity in the range of 96 to 99% (area). After the RP-HPLC stage the product is normally obtained in the form of a solution with a concentration of typically 1 to 15 % (w/w) of the peptide.

In order to obtain a dry final product which is suitable for the drug formulation the solution can either be subjected to lyophilization or precipitation techniques.

However, the procedures known in the art suffer from delivering a product in a form which is not freely flowable. Furthermore precipitation and lyophilization techniques are time consuming and need to be applied batchwise.

The object of the present invention therefore is to develop a process which is able to deliver a GLP-1 peptide drug in a freely flowable homogenous powder form and which is applicable on technical scale.

It was found that this object could be reached with the process of the present invention as outlined below.

The process for the production of a freely flowable homogenous powder form of a GLP-1 peptide analogue is characterized in that a solution of the peptide analogue in an aqueous organic solvent is subjected to a spray drying process and recovered in the form of a freely flowable homogenous powder. The solution of the peptide is directly obtained from the RP-HPLC stage or from a RP-LPLC (low-pressure liquid chromatography) or RP-MPLC (medium-pressure liquid chromatography).

The term "freely flowable" describes the property of spray dried GLP-1 peptide analogues to show favorable flow properties, i.e. the GLP-1 peptide is in a homogenous powder form with no tendency to form aggregates or lumps.

The term "GLP-1 peptide analogue" encompasses the natural human glucagon-like peptide-1 (GLP-1) analogues GLP-1 (7-37) and GLP-1 (7-36)NH_2 and synthetic analogues of the GLP-1 peptide (GLP-1 analogues).

Preferred GLP-1 analogues are the human GLP-1 analogue with the amino acid sequence according to SEQ ID No. 1:

His-Aib-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Lys-Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys-Aib-Arg-NH_2,

i.e. ($Aib^{8,35}$) GLP-1(7-36)NH_2, and further analogues as described in the PCT Publication WO 2000/34331. ($Aib^{8,35}$) GLP-1(7-36)NH_2 is most preferred. The short form designates an analogue formally derived from natural human GLP-1 (1-37) by deleting the amino acid residues Nos. 1 to 6, amidating at the C-terminus and substituting the naturally occurring amino acid residues in position 8 (Ala) and 35 (Gly) by α-aminoisobutyric acid (Aib).

Suitable analogues of the GLP-1 peptide can further be selected from GLP-1 (7-37), GLP-1 (7-36)NH_2, (Gly^8) GLP-1(7-37), (Gly^8) GLP-1(7-36), (Ser^{34})GLP-1 (7-37), (Val^8)GLP-1 (7-37), (Val^8,Glu^{22}) GLP-1 (7-37), (N-ε-(γ-Glu(N-α-hexadecanoyl)))-$Lys^{26}Arg^{34}$-GLP-1(7–37) (Liraglutide) and D-Ala^8Lys^{37}-(2-(2-(2-maleimidopropionamido(ethoxy)ethoxy)acetamide)) GLP-1 (7-37) (CJC-1131).

Still further analogues of the GLP-1 peptide can be the exendin analogues selected from exendin-3, exendin-4 (exenatide) having the amino acid sequence according to SEQ ID No. 2:

His-Gly-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Leu-Ser-Lys-Gln-Met-Glu-Glu-Glu-Ala-Val-Arg-Leu-Phe-Ile-Glu-Trp-Leu-Lys-Asn-Gly-Gly-Pro-Ser-Ser-Gly-Ala-Pro-Pro-Pro-Ser-NH_2,

exendin-4 acid, exendin-4 (1-30), exendin-4 (1-30) amide, exendin-4 (1-28), exendin-4 (1-28) amide, ^{14}Leu,^{25}Phe exendin-4 amide and ^{14}Leu,^{25}Phe exendin-4 (1-28) amide as well as AVE-0010, an exendin analogue having the amino acid sequence according to SEQ ID No. 3:

His-Gly-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Leu-Ser-Lys-Gln-Met-Glu-Glu-Glu- Ala-Val-Arg-Leu-Phe-Ile-Glu-Trp-Leu-Lys-Asn-Gly-Gly-Pro-Ser-Ser-Gly-Ala-Pro-Pro-Ser-Lys-Lys-Lys-Lys-Lys-Lys-NH_2.

The figures have the meaning as outlined below:

Fig.1: shows a flow chart of the spray drying process

Fig 2a: shows a scanning electron microscopy of a precipitated ($Aib^{8,35}$) GLP-1(7-36)NH_2

Fig 2b: shows a scanning electron microscopy of a spray dried ($Aib^{8,35}$) GLP-1(7-36)NH_2.

In a preferred embodiment of the invention the process is characterized in that the solution of the peptide analogue in an aqueous organic solvent is directly obtained from preparative HPLC and fed into the spray drying process.

The spray drying process comprises the steps of

a) feeding a solution of the peptide in an aqueous organic solvent from a feed tank (1) through a filter (2);

b) atomizing the filtered solution in a spray chamber (3) with the help of an atomizer (4);

c) mixing the atomized mixture with hot drying gas fed through inlet (5), thereby causing the solvent to evaporate and the peptide powder to precipitate; and

d) feeding the gas powder mixture into a cyclone (6) where the peptide can be collected as freely flowable homogenous powder.

Step a)

Step a) requires feeding a solution of the peptide in an aqueous organic solvent from a feed tank (1) through a filter (2).

As outlined above the solution of the peptide analogue in an aqueous organic solvent is preferably directly taken from the preparative HPLC (symbolized as "A" in Fig.1). In case mixtures of water with acetonitrile and THF are used as solvent in the purification step a subsequent concentration of the peptide solution has to be carried out by HPLC or, alternatively, by LPLC (low pressure liquid chromatography) or MPLC (medium pressure liquid chromatography) using a mixture of water with an aliphatic alcohol as solvent.

The aqueous organic solvent usually is a mixture of 20% to 80% w/w of water with 20% to 80% w/w of an aliphatic alcohol, preferably a mixture of 40% to 70% w/w of water with 30% to 60% w/w of an aliphatic alcohol.

The aliphatic alcohol can be selected from methanol, ethanol, n-propanol, 2-propanol, n-butanol, s-butanol or t-butanol, preferably methanol or ethanol.

The peptide content in the aqueous organic solvent as a rule ranges between 0.5% to 15% w/w, preferably between 1.0% to 10% w/w, more preferably between 0.5% to 8% w/w.

The solution of the peptide expediently contains a common buffer for pH stabilization. A suitable buffer is preferably selected from ammonium acetate, which can be dosed in the range of 0.05% to 0.25% w/w, or acetic acid, which can be dosed in the range of 0.05% to 1% w/w. More preferably, the buffer is acetic acid in an amount of 0.05 to 1% w/w.

The solution of the peptide is fed from a feed tank (1) through a filter (2) usually with a feed rate of 1 kg/h to 20 kg/h, preferably of 5 kg/h to 15 kg/h into the spray chamber (3). Depending on the size of the spray-dryer equipment used the feed rate can be consequently increased by a scale-factor of 5 to 10.

The temperature of the solution of the peptide can be selected between 5 °C to 35 °C.

The size of the filter (2) as a rule is in the range of 0.2 μm to 4.0 μm.

Step b)

Step b) requires atomizing the filtered solution in a spray chamber (3) with the help of an atomizer (4).

As a rule a rotary wheel atomizer is used. The atomizer speed is selected in a range of 10´000 rpm to 30´000 rpm.

Step c)

Step c) requires mixing the atomized mixture with hot drying gas feed through inlet (5), thereby causing the solvent to evaporate and the peptide powder to precipitate.

As "hot drying gas" nitrogen, carbon dioxide or air can be used. Preferred "hot drying gas" is nitrogen which can be applied at a temperature of 100 °C to 200 °C, preferably 110 °C to 140 °C.

The hot drying gas is fed into the spray chamber (3) with a feed rate of 300 kg/h to 500 kg/h. Depending on the size of the spray-dryer equipment used the feed rate can be consequently increased by a scale-factor of 5 to 10.

Step d)

Step d) requires feeding the gas powder mixture into a cyclone (6) where the peptide can be collected as freely flowable homogenous powder.

The gas fed into the cyclone (6) as a rule has a temperature of 50 °C to 150 °C, preferably 50 °C to 110 °C and more preferably 60 °C to 80 °C.

The peptide can be collected with equipment well known in the art such as in a bag housing (B).

Gas recovery

In a preferred embodiment of the invention the gas can be recovered according to the following gas recovery steps:

e) purifying the gas leaving the cyclone (6) with a filter (7);

f) condensing the aqueous organic solvent in a condenser (8);

g) heating the gas leaving the condenser (8) in a heater (9) and reintroducing the hot drying gas in the spray chamber (3).

Filter (7) is expediently connected to suitable equipment (C) for collecting fine particles withheld in the filter.

Condenser (8) is also connected to suitable equipment (D) for recovering the condensed solvent.

In the heater (9) the gas is again brought to the temperature for use as "hot drying gas" in the spray drying process.

Alternatively, only fresh gas can be used. The spray drying process is then operating in an "open cycle" mode versus operating in a "closed cycle" mode when the gas is recovered.

Product characteristics:

The peptide obtained from the spray drying process according to the present invention is as rule in a freely flowable homogenous powder form. The particles accordingly, when compared to a precipitated product, show a lower specific surface area and a lower bulk density. The majority of the particles of the spray dried product are much lower in diameter when compared to the precipitated product.

In a preferred embodiment the freely flowable homogenous powder of $(Aib^{8,35})hGLP\text{-}1_{(7\text{-}36)}\ NH_2$ is characterized by a specific surface area measured according to the BET method (ISO 9277) of 0.5 m^2/g to 5 m^2/g, preferably 0.5 m^2/g to 2.5 m^2/g i.e. values which are substantially lower than the values measured for the precipitated product.

The spray dried $(Aib^{8,35})hGLP\text{-}1_{(7\text{-}36)}NH_2$ is further characterized by a particle size distribution d_{90} (measured by laser scattering) of less than 200 μm, preferably less than 150 μm and more preferably less than 100 μm, which means that 90% of the particles have a particle size of less than 200 μm, preferably less than 100 μm. More than 60% of the precipitated peptide have a particle size exceeding 500 μm.

The mean size of the particles of the spray dried $(Aib^{8,35})hGLP\text{-}1_{(7\text{-}36)}NH_2$ as a rule is within a range of 10 to 60 μm, preferably within the range of 20 to 40 μm and more preferably within the range of 20 to 30 μm.

The processability parameter bulk density of the spray dried $(Aib^{8,35})hGLP\text{-}1(_{7\text{-}36})NH_2$ is as a rule less than 0.25 g/cm^3, preferably less than 0.2 g/cm^3, but can be adjusted to less than 0.1 g/cm^3 and thus is superior when compared to a precipitated product which shows values exceeding 0.3 to 0.4 g/cm^3.

It has been found that the bulk density and tapped density of the spray dried product can be adjusted depending on parameters such as the ratio of water to aliphatic alcohol in the aqueous organic solvent (feed solution), the concentration of the peptide and of the acetate in the feed solution and the pH value of the feed solution. With small amounts of acetate in the feed solution bulk densities of the spray dried $(Aib^{8,35})hGLP\text{-}1(_{7\text{-}36})NH_2$ of less than 0.1 g/cm^3 are obtained, whereas higher amounts of acetate in the feed solution lead to bulk densities of about 0.2 to 0.25 g/cm^3.

Thus, a spray dried powder of $(Aib^{8,35})hGLP\text{-}1(_{7\text{-}36})NH_2$ with a bulk density of less than 0.1 g/cm^3 is obtained by the process as described herein that is characterized in that the solution of the peptide (feed solution) contains less than 2 % w/w of $(Aib^{8,35})hGLP\text{-}1(_{7\text{-}36})NH_2$ and less than 0.15, preferably less than 0.10 % w/w of acetate. A spray dried powder of $(Aib^{8,35})hGLP\text{-}1(_{7\text{-}36})NH_2$ with a bulk density of about 0.20 to 0.25 g/cm^3 is obtained by the process as described herein that is characterized in that the solution of the peptide (feed solution) contains 7 to 8 % w/w of $(Aib^{8,35})hGLP\text{-}1(_{7\text{-}36})NH_2$ and acetate in the range of 0.4 to 0.6 % w/w.

The following examples shall illustrate the invention without limiting it.

Examples

Example A:

Preparation of the peptide

The crude peptide $(Aib^{8,35})GLP\text{-}1(7\text{-}36)NH_2$ can be prepared according to the method described in WO 2007/147816 by producing three fragments and coupling these fragments in solution.

Example B:

RP-HPLC Purification

Purification of the crude peptide is performed on a RP (reversed phase) stationary phase. Thus, the sorbent is RP material such as silica gel (e.g. Kromasil 100-16-C18) or acrylic ester macroreticular adsorbent (e.g. Amberchrom CG71M). The purification involves a 1st pass chromatographic purification at a pH of approximately 2, followed by a 2nd pass at a pH of approximately 9.

1st Chromatography:

Crude $(Aib^{8,35})GLP\text{-}1(7\text{-}36)NH_2$ is dissolved in water/acetonitrile/acetic acid (e.g. 90/9/1 v/v/v) and loaded onto a HPLC column (loading up to 20 g/L, bed depth approx. 25 cm) and the purification program (example for a column with a length of 45 cm is described in Table 1) is initiated. Fractions are collected and may be diluted with water or diluted ammonium hydroxide solution.

Table 1

Parameters and Purification Program of Chromatography 1

Parameter	Description
Sorbent	RP silica gel (Kromasil 100-16-C18)
Detection	UV
Eluent A	aqueous ammonium phosphate (approx. pH 2) / acetonitrile (80/20 v/v)
Eluent B	aqueous ammonium phosphate (approx. pH 2) / acetonitrile (60/40 v/v)
Column Flush	aqueous acetic acid or water / acetonitrile (25/75 v/v)

Program			
Time (min)	% organic solvent (by weight)	Flow (L/min)	Description
7	20	5 to 7	Column equilibration prior to injection
initial	20	5 to 7	Initial conditions after material is loaded on column
1	20-30	5 to 7	Initial gradient
40	30-35	5 to 7	Second gradient (product elutes)
4	70	5 to 7	Column flush

Proportions of A and B may be varied in order to achieve approximately the % organic solvent indicated in the purification program, corresponding to a minimal retention for the main peak (peptide $(Aib^{8,35})GLP\text{-}1(7\text{-}36)NH_2$). The event time, gradient and loading aspects may be varied in order to optimize the purification. The pooled fractions are further purified by the conditions of 2nd Chromatography.

2nd Chromatography:

The pooled diluted fractions from Chromatography 1 of $(Aib^{8,35})GLP\text{-}1(7\text{-}36)NH_2$ are loaded onto the HPLC column and the purification program (see examples for a 45 cm column in Table 2 and Table 3) is initiated. Fractions are collected and may be diluted with water or diluted acetic acid.

Table 2

Parameters and Purification Program of Chromatography 2 (Alternative 2a)

Parameter	Description		
Sorbent	RP silica gel (Kromasil 100-16-C18)		
Detection	UV		
Eluent C	Aqueous ammonium acetate (approx. pH 9) / ethanol (80/20 v/v)		
Eluent D	Aqueous ammonium acetate (approx. pH 9) / ethanol (35/65 v/v)		
Column Flush	Aqueous acetic acid / ethanol (10/90 v/v)		
Program			
Time (min)	% organic solvent (by weight)	Flow (L/min)	Description
initial	15	5 to 7	Initial conditions
1	15-35	5 to 7	Initial gradient
30	35-44	5 to 7	Second gradient (product elutes)

Proportions of C and D may be varied in order to achieve approximately the % organic solvent indicated in the purification program, corresponding to a minimal retention for the main peak (peptide ($Aib^{8,35}$) GLP-1(7-36)NH_2). The event time, gradient and loading aspects may be varied in order to optimize the purification.

The pooled fractions can be directly used in the precipitation process or the spray-drying process as described herein after.

Table 3

Parameters and Purification Program of Chromatography 2 (Alternative 2b)

Parameter	Description		
Sorbent	RP silica gel (Kromasil 100-10-C4)		
Detection	UV		
Eluent E	Aqueous ammonium acetate (approx. pH 7) / acetonitrile (80/20 v/v)		
Eluent F	Aqueous ammonium acetate (approx. pH 9) / acetonitrile / THF (25/60/15 v/v/v)		
Column Flush	Aqueous acetic acid / acetonitrile (10/90 v/v)		
Program			
Time (min)	% organic solvent (by weight)	Flow (L/min)	Description
10	12	5 to 7	Column equilibration prior to injection
initial	12	5 to 7	Initial conditions
10	12-28	5 to 7	Initial gradient
25	28	5 to 7	Isocratic hold
10	28-72	5 to 7	Column flush

Proportions of E and F are varied in order to achieve approximately the % organic solvent indicated in the purification program, corresponding to a minimal retention for the main peak (peptide ($Aib^{8,35}$) GLP-1(7-36)NH_2). The event time, gradient and loading aspects may be varied in order to optimize the purification.

The pooled fractions are collected and may be diluted with water or diluted acetic acid. They are then loaded directly onto the HPLC column for the following concentration step.

Concentration of (Aib^{8,35})GLP-1(7-36)NH_2:

The following step for concentration is optionally performed in case alternative 2a is used in the 2nd chromatography step. The pooled, diluted fractions from Chromatography 2 are loaded onto the column and equilibrated with a weak mobile phase (Initial Buffer in Table 4 or aqueous acetic acid/ethanol (85/15 v/v)). The buffer composition is changed to a strong mobile phase (Final Buffer in Table 4 or aqueous acetic acid/ethanol (20/80 v/v)) and (Aib^{8,35})GLP-1(7-36)NH_2 is collected as it elutes from the column.

Table 4

Concentration

Parameter	Description		
Sorbent	RP material (Kromasil 100-16-C18)		
Detection	UV		
Initial Buffer	Aqueous ammonium acetate (approx. pH 9)ethanol[1] (85/15 v/v)		
Final Buffer	Aqueous ammonium acetate (approx. pH 9)/ethanol[1] (20/80 v/v)		
Program			
Time (min)	% organic solvent (by weight)	Flow (L/min)	Description
initial	28	2 to 4	Initial conditions
1	28-35	2 to 4	Initial gradient
20	35-70	2 to 4	Second gradient (product elutes)

[1] Ethanol may be substituted by methanol.

The event time, gradient and loading aspects of the column may be varied in order to optimize the concentration. Several concentration runs are combined and the ethanol content of the (Aib^{8,35})GLP-1(7-36)NH_2 containing solution is determined (e.g. by GC).

Comparative Example C:

Precipitation:

In a suitable reactor 184 kg of methyl tert-butyl ether (MTBE) are mixed with 122 kg ethanol at a temperature of 24 to 26 °C. 36.7 kg of the purified solution of $(Aib^{8,35})$hGLP-1(7-36)NH_2 obtained from the preparative HPLC are added within 5 to 15 min. The mixture is heated to a temperature of 34 °C to 36 °C, stirred for 1 hour and then cooled to 24 °C to 26 °C. After filtration on a filter dryer (0.2 m^2) the cake is dried with nitrogen for 15 min and further dried under vacuum (less than 100 mbar) for 9 hours at 25 °C to 30 °C. The cake is then washed twice with 3.4 kg ethanol each and dried under vacuum (less than 100 mbar) for 19 hours at 25 °C. The obtained product is humidified by passing through the filter dryer from below damp nitrogen for 3 hours and dry nitrogen for 1 hour in an alternating manner until the ethanol content has been depleted to less than 1 %. Thereby the product takes up moisture and another drying cycle is necessary. By passing through the filter dryer from below dry nitrogen for 3 hours the moisture content is adjusted to approximately 6 %. 601 g of product (Yield: 92 % according to HPLC assay) are obtained (containing 97.9 % (area) $(Aib^{8,35})$hGLP-1(7-36)NH_2, 6.1 % H_2O, 0.73 % ethanol and 0.02 % MTBE).

Spray Drying Examples 1a and 1b

Spray drying conditions applied for example 1a and 1b:

Table 5

Feed solution composition:	0.8-1.7 % (w/w) of $(Aib^{8,35})$hGLP-1$(_{7-36})NH_2$ 58-79 % (w/w) water 20-40 % (w/w) ethanol´ 0.055-0.11 % (w/w) ammonium acetate
Feed solution quality:	97.9 $(Aib^{8,35})$hGLP-1$(_{7-36})NH_2$ (area %)
Feed Rate	9 -11 kg/h
Atomizing Mode	Rotary wheel atomizer
Atomizer speed:	15´000 RPM
Drying gas inlet temperature	135°C
Drying gas outlet temperature	76°C
Drying gas (nitrogen)	380 - 420 kg/h, closed cycle mode
In-situ" nitrogen purge"	no

Spray-dried $(Aib^{8,35})hGLP\text{-}1_{(7\text{-}36)}NH_2$ is of good quality without any new impurities related to thermal degradation (see following Table 6). It contains approximately 1-2 % (w/w) of ethanol, 4-5 % (w/w) of water as well as 3 % (w/w) of acetate (table 4).

Table 6:

Typical quality attributes of spray-dried $(Aib^{8,35})hGLP\text{-}1_{(7\text{-}36)}NH_2$

Quality Attribute	Spray-dried $(Aib^{8,35})hGLP\text{-}1_{(7\text{-}36)}NH_2$	
	Example 1a	Example 1b
Isolated Amounts	0.44 kg	0.15 kg
Assay - $(Aib^{8,35})hGLP\text{-}1_{(7\text{-}36)}NH_2$ (area %) - $(Aib^{8,35})hGLP\text{-}1_{(7\text{-}36)}NH_2$ (mass %)	 98.0% 87.6%	 97.6% 86.9%
Organic impurity (mass %) - RRT = 0.98 - RRT = 1.06 - RRT = 1.08 - Unspecified impurities - Total of all organic impurities	 0.39% 0.23% 0.25% 0.77% 1.6%	 0.39% 0.20% 0.27% 0.92% 1.8%
Residual solvents (% (w/w)) - Ethanol - other organic solvents	 1.1% < 0.01% each	 2.0% < 0.01% each
Water % (w/w))	4.8%	4.7%
Acetate (% (w/w))	2.9%	3.1%
Trifluoroacetate (% (w/w))	< 0.05%	< 0.05%
Triethylamine (% (w/w))	0.04%	0.04%
Ammonium (% (w/w))	< 0.02%	< 0.02%
Phosphate (% (w/w))	0.02%	0.02%

nd = not detected

Examples 2a to 2f:

Further small-scale optimization trials were preformed with some variations of the process parameters in order to minimize the amount of residual ethanol:

Table 7

Feed solution composition:	1.7 % (w/w) of $(Aib^{8,35})hGLP\text{-}1(_{7\text{-}36})NH_2$ 58 % (w/w) water 40 % (w/w) ethanol 0.15 % (w/w) ammonium acetate
Atomizing Mode	Rotary wheel atomizer
Atomizer speed:	20´000 RPM

Therefore a continuous "in-situ" nitrogen purge has been set up and a relative depletion of 23 to 28 % ethanol has been successfully achieved (table 8).

Table 8:

Process parameters and specific quality attributes of small-scale trials

Examples	Ex. 2a	Ex. 2b	Ex. 2c	Ex. 2d	Ex. 2e	Ex. 2f
- inlet temperature (°C) - outlet temperature (°C) - gas flow (kg/h)	135 65 350	120 70 400	120 65 400	135 65 350	135 65 350	135 65 350
"In-situ" nitrogen purge	no	no	no	no	yes	yes
Residual solvents (% (w/w)) - Ethanol - others each	 2.2% < 0.01%	 2.2% < 0.01%	 2.4% < 0.01%	 2.3% < 0.01%	 1.7% < 0.01%	 1.8% < 0.01%
Water (mass %)	5.6%	4.0%	4.0%	4.8%	n.a.	3.4%
Acetate (mass %)	2.7%	2.2%	2.3%	2.3%	n.a.	2.7%

Examples 3a and 3b:

Subsequent multi kilogram-scale batches were performed using following optimized parameters:

Table 9

Feed solution composition:	1.7-1.9 % (w/w) of $(Aib^{8,35})hGLP\text{-}1(_{7\text{-}36})NH_2$ 58 % (w/w) water 40 % (w/w) ethanol 0.15 % (w/w) ammonium acetate
Feed Rate	11-12 kg/h
Atomizing Mode	Rotary wheel atomizer
Atomizer speed:	20´000 RPM
Drying gas inlet temperature	135°C
Drying gas outlet temperature	65°C
Drying gas (nitrogen)	350 kg/h, closed cycle mode
In-situ" nitrogen purge"	continuous

The obtained quality attributes of two batches are depicted in table 10:

Table 10:

Quality attributes of large scale batches

Quality Attribute	Spray-dried $(Aib^{8,35})hGLP\text{-}1(_{7\text{-}36})NH_2$	
	Example 3a	Example 3b
Isolated Amounts	1. 9 kg	2.7 kg
Feed solution quality: $(Aib^{8,35})hGLP\text{-}1(7\text{-}36)NH_2$ (area %)	98.1%	97.7%
Assay $(Aib^{8,35})hGLP\text{-}1(7\text{-}36)NH_2$ (area %) $(Aib^{8,35})hGLP\text{-}1(7\text{-}36)NH_2$ (mass %)	 98.1% 88.6%	 97.3% 88.8%

Residual solvents (% (w/w)) - Ethanol - other organic solvents	 1.7% < 0.01% each	 1.6% < 0.01% each
Water (% (w/w))	4.5%	4.0%
Acetate (% (w/w))	2.7%	2.5%
Trifluoroacetate (% (w/w))	0.05%	< 0.05%
Isolation Capacity	0.16 kg/h	0.18 kg/h

Example 4:

160 kg of a solution containing 1.87% (w/w) $(Aib^{8,35})hGLP\text{-}1(_{7\text{-}36})NH_2$, 61% (w/w) water and 37% (w/w) ethanol is fed to a Niro SD-4-R-CC (Spraying chamber ø 1.2 x 0.75 m, capacity 8 kg H_2O/h). After about 15 hours, 2.73 kg of a fine powder (analytical values see example 3a above) are collected from the cyclone.

Table 11:

Comparison of solid state properties between precipitated and spray-dried $(Aib^{8,35})hGLP\text{-}1(_{7\text{-}36})NH_2$products.

A range obtained from various measurements is provided.

	Precipitated product (comparison)	Spray dried product (invention)
Specific surface area (BET) acording to ISO 9277 (m^2/g)/	14.9-18.8	0.8-1.4
Bulk Density (g/cm^3)	0.34-0.39	0.05-0.06
Tapped Density (ISO 3953) (g/cm^3)	0.38-0.43	0.08-0.11
Particle size distribution, particle size measured with laser scattering method (µm)	63% to90% of the particles >500 µm	d_{10}=11-12 d_{50}=33-38 d_{90}=67-86

Example 5:

Further optimization trials were performed to determine how the bulk density of the spray-dried powder can be adjusted in order to facilitate the down-stream processing. The results are shown in table 12. Examples 5a to 5f were carried out with different compositions of the feed solution, i.e. variations of the solvent ratio (ethanol to water), the concentration of $(Aib^{8,35})hGLP\text{-}1_{(7\text{-}36)}NH_2$ and the concentration of acetate in the feed solution resulting in different pH. The variations were integrated in the final preparative HPLC step in order to carry out the spray-drying of the preparative HPLC solution directly and without any further modification.

Table 12:

Evolution of the bulk density of large scale batches

	Ex. 5a	Ex. 5b	Ex. 5c	Ex. 5d	Ex. 5e	Ex. 5f
Feed solution composition						
- Water/Ethanol (% w/w)	59/41	62/38	61/39	57/43	62/38	58/42
- Acetate (% w/w)	0.09	0.09	0.09	0.49	0.56	0.53
- $(Aib^{8,35})hGLP\text{-}1_{(7\text{-}36)}NH_2$ (% w/w)	1.67	1.87	1.92	7.06	7.25	7.48
- pH	8.5	8.4	8.6	5.2	5.2	5.4
Bulk density (kg/L)	0.06	0.05	0.06	0.22	0.21	0.22
Dissolution time	2h55	2h58	2h55	1h51	1h41	2h01

Claims

1. Process for the production of a freely flowable homogenous powder form of a GLP-1 peptide analogue, wherein a solution of the peptide analogue in an aqueous organic solvent is subjected to a spray drying process and recovered in the form of a freely flowable homogenous powder.

2. Process according to claim 1, wherein the solution of the peptide in an aqueous organic solvent is obtained from preparative HPLC, LPLC or MPLC.

3. Process according to claim 1, wherein the solution of the peptide in an aqueous organic solvent is obtained from preparative HPLC.

4. Process according to claims 1 to 3, wherein the spray drying process comprises the steps of

a) feeding a solution of the peptide in an aqueous organic solvent from a feed tank (1) through a filter (2);

b) atomizing the filtered solution in a spray chamber (3) with the help of an atomizer (4);

c) mixing the atomized mixture with hot drying gas fed through inlet (5), thereby causing the solvent to evaporate and the peptide powder to precipitate; and

d) feeding the gas powder mixture into a cyclone (6) where the peptide can be collected as freely flowable homogenous powder.

5. Process according to claims 1 to 4, wherein the aqueous organic solvent is a mixture of 20% to 80% w/w of water with 20% to 80% w/w of an aliphatic alcohol.

6. Process according to claim 5, wherein the aliphatic alcohol is methanol or ethanol.

7. Process according to claims 1 to 6, wherein the solution of the peptide contains a buffer.

8. Process according to claims 1 to 6, wherein the solution of the peptide contains acetate.

9. Process according to claims 1 to 6, wherein the solution of the peptide contains 0.4 to 0.6 % w/w acetate.

10. Process according to claims 1 to 6, wherein the solution of the peptide contains less than 0.15 % w/w acetate.

11. Process according to claim 4, wherein in step a) of the spray drying process the solution of the peptide is fed from feed tank (1) through a filter (2) with a feed rate of 1 kg/h to 20 kg/h.

12. Process according to claim 11, wherein the solution of the peptide has a temperature of 5 °C to 35 °C.

13. Process according to any one of claims 4, 11 and 12, wherein in step a) of the spray drying process the size of the filter (2) is in the range of 0.2 µm to 4.0 µm.

14. Process according to claim 4, wherein in step b) of the spray drying process a rotary wheel atomizer is used.

15. Process according to claim 14, wherein the atomizer speed is selected in a range of 10´000 rpm to 30´000 rpm.

16. Process according to claim 4, wherein in step c) of the spray drying process nitrogen with a temperature of 100 °C to 200 °C is used as hot drying gas.

17. Process according to any one of claims 4 and 16, wherein the hot drying gas is fed with a feed rate of 300 kg/h to 500 kg/h.

18. Process according to claim 4, wherein in step d) of the spray drying process the gas fed into the cyclone (6) has a temperature of 50 °C to 110 °C.

19. Process according to claim 4, wherein the spray drying process in addition comprises the gas recovery steps of

e) purifying the gas leaving the cyclone (6) with a filter (7);

f) condensing the aqueous organic solvent in a condenser (8);

g) heating the gas leaving the condenser (8) in a heater (9) and reintroducing the hot drying gas in the spray chamber (3).

20. Process according to any one of claims 1 to 19, wherein the GLP-1 peptide analogue is selected from the group consisting of GLP-1 (7-37), GLP-1 (7-36)NH_2, (Gly^8) GLP-1(7-37), (Gly^8) GLP-1(7-36), (Ser^{34})GLP-1 (7-37), (Val^8)GLP-1 (7-37), (Val^8,Glu^{22}) GLP-1 (7-37), ($Aib^{8,35}$)hGLP-1($_{7-36}$)NH_2., (N-ε-(γ-Glu(N-α-hexadecanoyl)))-$Lys^{26}Arg^{34}$-GLP-1(7–37), D-Ala^8Lys^{37}-(2-(2-(2-maleimidopropionamido(ethoxy)ethoxy)acetamide)) GLP-1 (7-37), exendin-3, exendin-4, exendin-4 acid, exendin-4 (1-30), exendin-4 (1-30) amide, exendin-4 (1-28), exendin-4 (1-28) amide, ^{14}Leu,^{25}Phe exendin-4 amide and ^{14}Leu,^{25}Phe exendin-4 (1-28) amide and AVE-0010.

21. Process according to any one of claims 1 to 19, wherein the GLP-1 peptide analogue is the ($Aib^{8,35}$)hGLP-1(7-36)NH_2.

22. GLP-1 peptide analogue obtainable with a process according to claims 1 to 19.

23. Freely flowable homogenous powder form of ($Aib^{8,35}$)hGLP-1(7-36)NH_2.

24. Freely flowable homogenous powder form of ($Aib^{8,35}$)hGLP-1(7-36)NH_2 according to claim 23, wherein the particles have a specific surface area (BET) of 0.5 m^2/g to 5 m^2/g.

25. Freely flowable homogenous powder form of ($Aib^{8,35}$)hGLP-1(7-36)NH_2 according to claims 22 and 23, wherein 90% of the particles have a diameter of less than 200 μm.

26. The novel processes and novel powder forms as described herein before.

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