PCT/EP2013/058619
Sanofi-Aventis Deutschland GmbH
52972P WO-1/WWAWil

New Claims 1-16
(clean version)

1. A method for the quantitative determination of an impurity present in a peptide product composition, comprising the steps:
   (a) providing a peptide product composition comprising a peptide product, which is an exendin peptide product comprising the amino acid sequence S-S-G-A and an unknown amount of at least one impurity which is a Di-Ser(33)-peptide and/or a Di-Ala(35)-peptide, wherein said impurity cannot be separated from the peptide product or another ingredient of the composition by a chromatographic procedure and wherein said impurity co-elutes or substantially co-elutes with the desired peptide product after the chromatographic separation procedure,
   (b) providing at least one sample of the peptide product composition without said impurity added and at least one further sample of the peptide product composition with a known amount of said impurity added,
   (c) quantitatively determining said impurity in said sample from step (b) by mass spectrometry, and
   (d) calculating the amount of said impurity in the peptide product composition based on the results of (c).

2. A method for the quantitative determination of an impurity present in a peptide product composition, comprising the steps:
   (a) providing a peptide product composition comprising a peptide product , which is an exendin peptide product comprising the amino acid sequence S-S-G-A and an unknown amount of at least one impurity which is a Di-Ser(33)-peptide and/or a Di-Ala(35)-peptide, wherein said impurity cannot be separated from the peptide product or another ingredient of the composition by a chromatographic procedure,
   (b) providing at least three samples of the peptide product composition, wherein a first sample comprises the peptide product composition without said impurity

added, and wherein at least two further samples comprise the peptide product composition each with a different known amount of said impurity added,

(c) quantitatively determining said impurity in said samples from step (b) by mass spectrometry, and

(d) calculating the amount of said impurity in the peptide product composition based on the results of (c).

3. The method of claim 1 or 2, wherein the peptide product has a length of from 5-100 amino acids.

4. The method of any one of claims 1-3, wherein the peptide product has been chemically synthesized, particularly by a solid phase synthesis procedure, or produced by recombinant DNA processes.

5. The method of any one of claims 1-4, wherein the peptide product composition is a pharmaceutical formulation or a composition intended for the manufacture of a pharmaceutical formulation.

6. The method of any one of claims 1-5, wherein the exendin peptide product is Lixisenatide (AVE0010).

7. The method of any one of claims 1-6, wherein the impurity cannot be quantitatively separated from the peptide product or from another ingredient of the composition by an HPLC procedure, particularly by a Reverse Phase HPLC procedure.

8. The method of any one of claims 1-7, wherein the peptide product comprises unknown amounts of at least 2 impurities which cannot be quantitatively separated from the peptide product or from another ingredient of the composition by a chromatographic procedure.

9. The method of any of claims 1-8, wherein the impurity is added to at least one peptide product composition sample from a stock preparation, preferably from at least two stock preparations comprising different concentrations of the impurity.

10. The method of any one of claims 1-9, wherein at least 3 or 4 further samples with different known amounts added impurity are provided and subjected to mass spectrometry determination.

11. The method of any one of claims 1-10, wherein the mass spectrometry is high resolution mass spectrometry, e.g. comprising Fourier Transform mass spectrometry.

12. The method of any one of claims 1-11, wherein the calculation comprises a linear regression analysis.

13. The method of claim 12, wherein the calculation is carried out according to the equation:

$$y = ax+b$$

wherein a = slope

y = determined peak area of the impurity in a sample

x = added amount of the impurity in a sample

b = intercept

and the unknown amount of the impurity x_t is obtained as follows:

$$x_t = b \bullet a^{-1}$$

14. A reagent kit for determining the amount of impurities in a Lixisenatide (AVE 0010) product composition, comprising:

(i) at least one stock preparation of Di-Ser(33)-AVE0010 (SEQ ID NO:2)

H-G-E-G-T-F-T-S-D-L-S-K-Q-M-E-E-E-A-V-R-L-F-I-E-W-L-K-N-G-G-P-S-S-S-G-A-P-P-S-K-K-K-K-K-K-NH_2

and/or

(ii) at least one stock preparation of Di-Ala(35)-AVE0010 (SEQ ID NO:3)

H-G-E-G-T-F-T-S-D-L-S-K-Q-M-E-E-E-A-V-R-L-F-I-E-W-L-K-N-G-G-P-S-S-G-A-A-P-P-S-K-K-K-K-K-K-NH_2.

15. A method for the quality control of a composition comprising an exendin peptide product comprising the amino acid sequence S-S-G-A, preferably of a composition comprising a Lixisenatide (AVE0010) product, comprising quantitatively determining the amount of a Di-Ser(33)-peptide, e.g. Di-Ser(33)-AVE0010 and/or a Di-Ala(35)-peptide, e.g. Di-Ala(35)-AVE0010, in said composition.

16. The method of claim 15, wherein the quantitative determination is carried out according to the method of any one of claims 1-13.

Claims

1. A method for the quantitative determination of an impurity present in a peptide product composition, comprising the steps:

   (a) providing a peptide product composition comprising a peptide product, which is an exendin peptide product comprising the amino acid sequence S-S-G-A and an unknown amount of at least one impurity which is a Di-Ser(33)-exendin peptide and/or a Di-Ala(35)-exendin peptide, wherein said impurity cannot be separated from the peptide product or another ingredient of the composition by a chromatographic procedure and wherein said impurity co-elutes or substantially co-elutes with the desired peptide product after the chromatographic separation procedure,

   (b) providing at least one sample of the peptide product composition without said impurity added and at least one further sample of the peptide product composition with a known amount of said impurity added,

   (c) quantitatively determining said impurity in said sample from step (b) by mass spectrometry, and

   (d) calculating the amount of said impurity in the peptide product composition based on the results of (c).

2. A method for the quantitative determination of an impurity present in a peptide product composition, comprising the steps:

   (a) providing a peptide product composition comprising a peptide product , which is an exendin peptide product comprising the amino acid sequence S-S-G-A and an unknown amount of at least one impurity which is a Di-Ser(33)-exendin peptide and/or a Di-Ala(35)-exendin peptide, wherein said impurity cannot be separated from the peptide product or another ingredient of the composition by a chromatographic procedure,

   (b) providing at least three samples of the peptide product composition, wherein a first sample comprises the peptide product composition without said impurity added, and wherein at least two further samples comprise the peptide product composition each with a different known amount of said impurity added,

   (c) quantitatively determining said impurity in said samples from step (b) by mass spectrometry, and

(d) calculating the amount of said impurity in the peptide product composition based on the results of (c).

3. The method of claim 1 or 2, wherein the peptide product has a length of from 5-100 amino acids.

4. The method of any one of claims 1-3, wherein the peptide product has been chemically synthesized, particularly by a solid phase synthesis procedure, or produced by recombinant DNA processes.

5. The method of any one of claims 1-4, wherein the peptide product composition is a pharmaceutical formulation or a composition intended for the manufacture of a pharmaceutical formulation.

6. The method of any one of claims 1-5, wherein the exendin peptide product is Lixisenatide (AVE0010).

7. The method of any one of claims 1-6, wherein the impurity cannot be quantitatively separated from the peptide product or from another ingredient of the composition by an HPLC procedure, particularly by a Reverse Phase HPLC procedure.

8. The method of any one of claims 1-7, wherein the peptide product comprises unknown amounts of at least 2 impurities which cannot be quantitatively separated from the peptide product or from another ingredient of the composition by a chromatographic procedure.

9. The method of any of claims 1-8, wherein the impurity is added to at least one peptide product composition sample from a stock preparation, preferably from at least two stock preparations comprising different concentrations of the impurity.

10. The method of any one of claims 1-9, wherein at least 3 or 4 further samples with different known amounts added impurity are provided and subjected to mass spectrometry determination.

11. The method of any one of claims 1-10, wherein the mass spectrometry is high resolution mass spectrometry, e.g. comprising Fourier Transform mass spectrometry.

12. The method of any one of claims 1-11, wherein the calculation comprises a linear regression analysis.

13. The method of claim 12, wherein the calculation is carried out according to the equation:

$$y = ax+b$$

wherein a = slope

y = determined peak area of the impurity in a sample

x = added amount of the impurity in a sample

b = intercept

and the unknown amount of the impurity x_t is obtained as follows:

$$x_t = b \bullet a^{-1}$$

14. A reagent kit for determining the amount of impurities in a Lixisenatide (AVE 0010) product composition, comprising:
(i) at least one stock preparation of Di-Ser(33)-AVE0010 (SEQ ID NO:2)
H-G-E-G-T-F-T-S-D-L-S-K-Q-M-E-E-E-A-V-R-L-F-I-E-W-L-K-N-G-G-P-S-S-S-G-A-P-P-S-K-K-K-K-K-K-NH_2
and/or
(ii) at least one stock preparation of Di-Ala(35)-AVE0010 (SEQ ID NO:3)
H-G-E-G-T-F-T-S-D-L-S-K-Q-M-E-E-E-A-V-R-L-F-I-E-W-L-K-N-G-G-P-S-S-G-A-A-P-P-S-K-K-K-K-K-K-NH_2.

15. A method for the quality control of a composition comprising an exendin peptide product comprising the amino acid sequence S-S-G-A, comprising quantitatively

determining the amount of a Di-Ser(33)-exendin peptide and/or a Di-Ala(35)-exendin peptide, in said composition.

16. The method of claim 15, wherein the quantitative determination is carried out according to the method of any one of claims 1-13.

17. The method of claim 15, wherein preferably of a composition comprising a Lixisenatide (AVE0010) product, Di-Ser(33)-AVE0010 and Di-Ala(35)-AVE0010.