What is claimed

1. A ~~M~~method for separating peptide aggregates and fragments from solutions containing the target peptide, comprising the steps of

(a) providing the sample containing the target peptide;

(b) contacting the sample with a hydrophobic chromatography material for a suitable period of time and adsorbing peptides comprising the target peptide and optionally peptide aggregates and fragments in the sample, and wherein the hydrophobic chromatography material, is particulate, which is made of cross-linked polymer, selected from the group consisting of ethylstyrene, poly(ethyl)styrene-divinylbenzene, and poly(ethyl)styrene-divinylbenzene ethyleneglycol-dimethylacrylate resin and wherein the particulate hydrophobic chromatography material is a resin, which is composed of a cross-linked polymer consisting of styrene and divinylbenzene in a ratio 98 : 2 up to 10 : 90 % by weight or of polystyrene, which is cross-linked with copolymer of divinylbenzene and ethylenglycol-di-methacrylate in a ratio of 98 : 2 up to 10 : 90 % by weight, ~~or which is made of cross-linked polymer, selected from the group , ethylstyrene, poly(ethyl)styrene-divinylbenzene, and poly(ethyl)styrene-divinylbenzene ethyleneglycol-dimethylacrylate resin for a suitable period of time and adsorbing the peptides,~~

(c) recovering the target peptide from the hydrophobic chromatography material by use of different solvent compositions~~,~~ and ~~whereby~~ thereby separating aggregated peptides and peptide fragments from the target peptide and wherein organic solvents~~,~~ selected from the group consisting of ethanol, 1-propanol and dipropylenglycol~~,~~ are used in the different solvent composition to selectively desorb the adsorbed ~~substances~~ peptides from the hydrophobic chromatography material~~;~~ ~~and thereby separating aggregated peptides and peptide fragments from the target peptide and~~

~~where either~~ wherein the separation of peptide aggregates is processed after a target molecule refolding step and,

~~or~~

wherein after contacting the sample with a hydrophobic chromatography material for a suitable period of time and adsorbing the peptides, the

loaded chromatography material is subjected to post peptide refolding solutions for selectively reducing the level of aggregated substances for a suitable period of time, and ~~before separating aggregated peptides and peptide fragments from the target peptide~~
wherein in step b) the hydrophobic chromatographic materiel is exposed to 30-100 mg of target peptide per ml of packed bed, at a flow rate in the range of 150-1000 cm/min.

~~2. The method of claim 1, wherein the hydrophobic chromatography material, is particulate.~~

~~3.~~2. ~~The~~ A method according to ~~one or more of the claims 1 or 2~~ claim 1, wherein the particulate hydrophobic chromatographic separation material has mean particle diameters in the range of 10 µm to 600 µm~~, preferably in the range of 20 µm to 150 µm, most preferably in the range of 20 µm to 63 µm~~.

~~4.~~3. ~~The~~ A method according to ~~one or more of the claims 1 to 3~~ claim 1, wherein the particulate hydrophobic chromatographic separation material consists of hydrophobic porous polymer beads having pore sizes in the range of 4 – 500 nm~~, preferably in the range of 10 – 30 nm, most preferred in the range of 13 nm to 25 nm~~.

~~5.~~4. ~~The~~ A method according to ~~one or more of the claims 1 to 4~~ claim 1, wherein in step b) an aqueous solution containing the target peptide, having a pH value in the range of 2 – 11, ~~preferably in a range of 3 – 8~~ and a conductivity in the range of 1 – 150 mS/cm, ~~preferably in the range of 2 – 50 mS/cm,~~ is contacted with a hydrophobic chromatography material.

~~6. The method according to one or more of the claims 1 to 5, wherein in step b) the hydrophobic chromatographic material is exposed to 30-100 mg of target peptide per ml of packed bed, preferably to 50-80 mg of target peptide per ml of packed bed at a flow rate in the range of 150 – 1000 cm/min, preferably in the range of 300 – 900 cm/min.~~

~~7.~~5. ~~The~~ A method according to ~~one or more of the claims 1 to 6~~ claim 1, wherein in step c) the hydrophobic chromatographic material is exposed to an aqueous solution comprising organic solvent in direct or gradient manner, whereby depending on the concentration of the contained organic solvent, ~~a partial~~ separation ~~between~~ of the aggregated peptides and peptide fragments from the target peptide is achieved.

~~8.~~6. ~~The~~ A method according to ~~one or more of the claims 1 to 7~~ claim 1, wherein in step c) a selective desorption and separation of the bound components is achieved using various ratios of organic solvents contained in an aqueous mixture.

~~9.~~7. ~~The~~ A method according to ~~one or more of the claims 1 to 8~~ claim 1, wherein the separation and purification sequence includes a treatment with an ion exchange resin.

~~10.~~8. ~~The~~ A method according to ~~one or more of the claims 1 to 9~~ claim 1, wherein the separation and purification is carried out in a bind and elute ~~or flow-through~~ mode, whereby the flow velocity is adjusted in the range of 150 cm/min – 1000 cm/min~~, and especially between 300 - 900cm/min~~.

~~11.~~9. ~~The~~ A method according to ~~one or more of the claims 1 to 10~~ claim 1, wherein in step b) the sample is contacted with a hydrophobic chromatography material in form of polymer beads in a liquid chromatography column having a diameter ranging from 1 to 100 cm, ~~preferably in the range of 5 to 50 cm,~~ and where the column is operated at pressures up to 100 bar~~, and preferably at pressure ranging from 0,2 to 80 bar~~.

~~12.~~10. ~~The~~ A method according to ~~one or more of the claims 1 to 10~~ claim 1, wherein the sample is contacted with a hydrophobic chromatography material in form of polymer beads in a liquid chromatography column having a diameter in the range of 10 to 50 cm and where the column is operated at pressures in the range of 0~~,~~.2 to 80 bar.

11. A method according to claim 1, wherein in step a) a crude insulin solution originating from E. coli expression system is provided.

12. The method according to claim 3, wherein the hydrophobic porous polymer beads have a pore size in the range of 10-30 nm.

13. The method according to claim 3, wherein the hydrophobic porous polymer beads have a pore size the range of 13 nm to 25 nm.

14. The method according to claim 4, wherein the aqueous solution containing the target peptide has a pH value in the range of 3-8 and a conductivity in the range 2-50 mS/cm.

15. The method according to claim 1, wherein in step b) the hydrophobic chromatographic material is exposed to 50-80 mg of target peptide per ml of packed bed at a flow rate in the range of 300-900 cm/min.

16. The method according to claim 8, whereby the flow velocity is adjusted in the range of 300-900 cm/min.

17. The method according to claim 9, wherein the liquid chromatography column has a diameter ranging from 5 to 50 cm, and where the column is operated at a pressure of 0.2 to 80 bar.

18. The method according to claim 2, wherein the particulate hydrophobic chromatographic separation material has mean particle diameters in the range of 20 µm to 150 µm.

19. The method according to claim 2, wherein the particulate hydrophobic chromatographic separation material has mean particle diameters in the range of 20 µm to 63 µm.