CLAIMS:

1. A method of identifying polysorbate in a sample containing protein comprising:

(i) precipitating the protein by exposing said sample to an organic protic polar solvent or an organic aprotic polar solvent,

(ii) separating the protein from the precipitated sample by centrifuging the precipitated sample to pellet the protein or peptide and obtaining a liquid supernatant,

(iii) separating the polysorbates by subjecting the supernatant to chromatography, wherein the chromatography comprises applying the supernatant to a stationary phase column comprising an immobilised cyano group, and eluting the bound polysorbates using a mobile phase composition gradient, and

(iv) detecting the separated polysorbates using a chromophore-lacking detector to identify polysorbate.

2. The ~~A~~ method according to claim 1, wherein ~~said~~ the method (i) identifies intact polysorbate and/or degraded polysorbate products; and/or (ii) comprises quantitating polysorbate.

~~3. A method according to claims 1-2, which further comprises quantitating polysorbate.~~

43. ~~A~~ The method according to any one of claims 1~~-~~ to 3, wherein the protein sample comprises (i) an antibody, a monoclonal antibody, or a fragment thereof; and/or (ii) acetate or citrate buffer; and/or (iii) protein at a concentration of from about 5mg/ml to about 300mg/ml.

~~5. A method according to any of the preceding claims, wherein said protein is a monoclonal antibody or a fragment thereof.~~

~~6~~4. ~~A~~ The method according to any one of the preceding claims, wherein ~~said~~ the method detects polysorbate selected from any one of PS80, PS60, PS40 and PS20.

~~7~~5. ~~A~~ The method according to any one of the preceding claims, wherein the chromophore lacking detector is a charged aerosol detector (CAD).

~~8. A method according to any of the preceding claims, wherein the protein sample comprises acetate or citrate buffer.~~

~~9. A method according to any of the preceding claims, wherein said protein in said sample is present at a concentration of from about 5mg/ml to about 300mg/ml .~~

~~10~~6. ~~A~~ The method according to any one of the preceding claims, wherein said protein precipitation is performed using a solvent selected from methanol, isopropyl alcohol (IPA), THF or acetone.

~~11~~7. ~~A~~ The method according to any one of the preceding claims, wherein said separation of polysorbate is performed (i) using a reverse phase HPLC column which comprises an immobilised cyano group and/or (ii) using a heated column which has a temperature of between about 20ºC and about 80ºC.

~~12~~8. ~~A~~ The method according to claim ~~11~~7, wherein said column is a silica column which has a pore size of ≥ 80 Angstroms.

~~13~~9. ~~A~~ The method according to any one of the preceding claims, wherein said elution is performed using a gradient separated mobile phase which is a mobile phase consisting of buffer A and buffer B wherein buffer A is a mixture of 0.1% Trifluoroacetic acid (TFA) in H_2O and buffer B is methanol or acetonitrile.

~~14. A method according to any of the preceding claims, wherein said separation of polysorbate is performed using a heated column which has a temperature of between about 20°C and about about 80°C.~~

~~15~~10. ~~A~~ The method according to claim 1 comprising:

(i) precipitating the protein by exposing said sample to methanol or IPA,

(ii) separating the protein from the precipitated sample by centrifuging the precipitated sample to pellet the protein and obtaining a liquid supernatant,

(iii) separating the polysorbates by subjecting the supernatant to reverse phase HPLC on a silica column with pore size of about 300 Angstroms and which comprises an immobilised cyano group and eluting using a mobile phase composition gradient consisting of A and B wherein A is a mixture of 0.1% Trifluoroacetic acid (TFA) in H_2O and B is methanol or acetonitrile,

(iv) detecting the separated polysorbates using a charged aerosol detector (CAD) to identify polysorbate.

~~16~~11. A method for identification of a protein sample wherein said identified protein sample(s) contains from about 10ppm-to about 5000ppm of intact polysorbate, and which comprises the following steps:

(a) measuring polysorbate in said samples, comprising the following steps

(i) precipitating the protein by exposing said sample to an organic protic polar solvent or an organic aprotic polar solvent,

(ii) separating the protein from the precipitated sample by centrifuging the precipitated sample to pellet the protein or peptide and obtaining a liquid supernatant,

(iii) separating the polysorbates by subjecting the supernatant to chromatography, wherein the chromatography comprises applying the supernatant to a stationary phase column comprising an immobilised cyano group, and eluting the bound polysorbates using a mobile phase composition gradient, and

(iv) detecting the separated polysorbates using a chromophore-lacking detector to identify polysorbate;

(b) ~~Identifying~~ identifying the protein sample(s) from (a) which have levels of intact polysorbate which are between about 10ppm to about 5000ppm,

(c) ~~Isolation~~ isolation and recovery of said protein(s) identified in step (b).

~~17~~12. ~~A~~ The method according to claim ~~16~~11, wherein said isolated protein sample in step (c) contains from about 10ppm to about 700ppm of intact polysorbate.

~~18~~13. ~~A~~ The method according to claim~~s~~ ~~16~~ 11 or ~~17~~12, wherein said polysorbate is PS80.

~~19~~14. ~~A~~ The method according to any one of claims ~~16~~ 11 ~~-~~to ~~18~~13, wherein said chromophore lacking detector is a charged aerosol detector (CAD).

~~20~~15. A protein or peptide polysorbate obtained or obtainable according to the method of claims ~~16~~11-~~19~~14.

~~21. A protein according to claim 20, which is a monoclonal antibody or a fragment thereof.~~

~~22. A protein according to claims 20-21, for use in medicine.~~

~~23. Use of a protein according to claims 20-21, for therapy or diagnosis of a human subject.~~

~~24. Use of a protein according to claims 20-21, in the manufacture of a medicament to treat disease in a human subject.~~

~~25. A pharmaceutical formulation comprising a protein according to claims 20-21, in combination with one or more pharmaceutically acceptable excipients.~~