CLAIMS:

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

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

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

(iii) separating subspecies of the polysorbates by subjecting the liquid supernatant to chromatography, wherein the chromatography comprises applying the liquid supernatant to a high performance liquid chromatography (HPLC) column comprising an immobilised cyano group, and eluting the polysorbates using a mobile phase composition gradient, and

(iv) detecting the separated subspecies of the polysorbates using a charged aerosol detector (CAD).

2. The method according to claim 1, wherein the method (i) identifies intact polysorbate and/or degraded polysorbate products; and/or (ii) comprises quantitating the separated subspecies of the polysorbates.

3. The method according to any one of claims 1 to 3, wherein the protein 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 5mg/ml to 300mg/ml.

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

5. The method according to any one of the preceding claims, wherein said organic polar solvent is selected from methanol, isopropyl alcohol (IPA), THF and acetone.

6. 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 from 20ºC to 80ºC.

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

8. 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.

9. The method according to claim 1, wherein:

(i) the organic polar solvent is methanol or IPA, and

(ii) the chromatography is reverse phase HPLC , the HPLC column is a silica column with pore size of about 300 Angstroms, and the mobile phase composition gradient consists of A and B, wherein A is a mixture of 0.1% Trifluoroacetic acid (TFA) in H_2O and B is methanol or acetonitrile.

10. A method for identification of a protein sample that contains from 10ppm to 5000ppm of intact polysorbate, wherein the method comprises:

(a) measuring polysorbates in a sample comprising a protein, comprising:

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

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

(iii) separating subspecies of the polysorbates by subjecting the liquid supernatant to chromatography, wherein the chromatography comprises applying the liquid supernatant to a high performance liquid chromatography (HPLC) column comprising an immobilised cyano group, and eluting the polysorbates using a mobile phase composition gradient, and

(iv) detecting the separated subspecies of the polysorbates using a charged aerosol detector (CAD) and quantitating intact polysorbates;

(b) identifying the sample from (a) which contains from 10ppm to 5000ppm of the intact polysorbates,

(c) isolation and recovering said sample identified in step (b).

11. The method according to claim 10, wherein said sample isolated and identified in step (c) contains from 10ppm to 700ppm of intact polysorbate.

12. The method according to claim 10 or 11, wherein said polysorbate is PS80.