CLAIMS

1. A method for the determination of polysorbate in a protein-containing sample, comprising the steps:
   (a) subjecting the sample to alkaline hydrolysis;
   (b) neutralizing the sample after alkaline hydrolysis;
   (c) optionally removing denaturated precipitate from the neutralized sample;
   (d) adding an aqueous mixture of a thiocyanatometal complex to the optionally filtered sample to form a sorbitan polyoxyethylenethiocyanatometal complex;
   (e) extracting said sorbitan polyoxyethylenethiocyanatometal complex formed in step (d) into a non-water miscible organic solvent;
   (f) measuring the absorbance of the extract obtained in step (e) to quantify the amount of said sorbitan polyoxyethylenethiocyanatometal complex formed in step (d); and
   (g) calculating the amount of the polysorbate contained in the sample from the amount of said sorbitan polyoxyethylenethiocyanatometal complex determined in step (f).

2. The method according to claim 1, wherein the polysorbate is polyoxyethylene sorbitan monooleate (polysorbate 80).

3. The method according to claim 2, wherein the polysorbate 80 originates from animals.

4. The method according to claim 2, wherein the polysorbate 80 originates from plants.

5. The method according to any one of claims 1 to 4, wherein the thiocyanatometal complex added in step (d) is an aqueous mixture of $Co(NO_3)_2 \cdot 6\ H_2O$ and NH_4SCN and the sorbitan polyoxyethylenethiocyanatometal complex formed is a sorbitan polyoxyethylenethiocyanatocobaltate(II) complex.

6. The method according to claim 5, wherein said aqueous mixture contains about 3% (w/v) $Co(NO_3)_2 \cdot 6\ H_2O$ and about 20% (w/v) NH_4SCN.

7. The method according to any one of claims 1 to 6, wherein the sample further contains isooctylphenol polyoxyethylene ether (Triton™ X-100).

8. The method according to any one of claims 1 to 7, wherein the sample further contains tri-n-butyl phosphate (TNBP).

9. The method according to any one of claims 1 to 8, wherein alkaline hydrolysis in step (a) comprises hydrolysis with an alkaline agent, selected from the group consisting of NaOH, KOH, LiOH, $Ba(OH)_2$, $Sr(OH)_2$, $Ca(OH)_2$, tetramethylammonium hydroxide, tetraethylammonium hydroxide, tetrapropylammonium hydroxide, tetrabutylammonium hydroxide, and homologous or heterologous substituted quaternary alkyl- and cycloalkylammonium hydroxides.

10. The method according to any one of claims 1 to 9, wherein alkaline hydrolysis in step (a) is carried out at a temperature in the range of about 80°C to about 100°C for at least 15 minutes.

11. The method according to any one of claims 1 to 10, wherein alkaline hydrolysis in step (a) comprises hydrolysis with at least 3 N NaOH at a temperature of about 95°C to about 100°C for at least 45 minutes.

12. The method according to any one of claims 1 to 11, wherein the filter used in step (c) does not bind polyoxyethylene sorbitan.

13. The method according to any one of claims 1 to 12, wherein the non-water miscible organic solvent used in step (e) is selected from the group consisting of methylene chloride, chloroform, *o*-dichlorobenzene, bromoform, and trichloroethylene.

14. The method according to any one of claims 1 to 13, wherein the non-water miscible organic solvent is methylene chloride.

15. The method according to any one of claims 1 to 14, wherein the absorbance of the extract obtained in step (e) is measured in step (f) at about 324 nm.

16. The method according to any one of claims 1 to 15, further comprising after step (a) and before step (b) the step:
(a2) cooling the sample after alkaline hydrolysis.

17. The method according to any one of claims 1 to 16, further comprising after step (b) and before step (c) the step:
(b2) keeping the sample at room temperature for at least 30 minutes to allow the formation of denatured precipitate.

CLAIMS

1. A method for the determination of polysorbate in a protein-containing sample, comprising the steps:

(a) subjecting the sample to alkaline hydrolysis;

(b) neutralizing the sample after alkaline hydrolysis;

(c) optionally removing denaturated precipitate from the neutralized sample;

(d) adding an aqueous mixture of a thiocyanatometal complex to the optionally filtered sample to form a sorbitan polyoxyethylenethiocyanatometal complex;

(e) extracting said sorbitan polyoxyethylenethiocyanatometal complex formed in step (d) into a non-water miscible organic solvent;

(f) measuring the absorbance of the extract obtained in step (e) to quantify the amount of said sorbitan polyoxyethylenethiocyanatometal complex formed in step (d); and

(g) calculating the amount of the polysorbate contained in the sample from the amount of said sorbitan polyoxyethylenethiocyanatometal complex determined in step (f),

wherein alkaline hydrolysis in step (a) comprises hydrolysis with an alkaline agent, selected from the group consisting of NaOH, KOH, LiOH, Ba(OH)2, Sr(OH)2, Ca(OH)2, tetramethylammonium hydroxide, tetraethylammonium hydroxide, tetrapropylammonium hydroxide, tetrabutylammonium hydroxide, and homologous or heterologous substituted quaternary alkyl- and cycloalkylammonium hydroxides, and

wherein alkaline hydrolysis in step (a) is carried out at a temperature in the range of about 80°C to about 100°C for at least 45 minutes.

2. The method according to claim 1, wherein the polysorbate is polyoxyethylene sorbitan monooleate (polysorbate 80).

3. The method according to claim 2, wherein the polysorbate 80 originates from animals.

4. The method according to claim 2, wherein the polysorbate 80 originates from plants.

5. The method according to any one of claims 1 to 4, wherein the thiocyanatometal complex added in step (d) is an aqueous mixture of $Co(NO_3)_2 \cdot 6 H_20$ and NH_4SCN and the sorbitan polyoxyethylenethiocyanatometal complex formed is a sorbitan polyoxyethylenethiocyanatocobaltate(II) complex.

6. The method according to claim 5, wherein said aqueous mixture contains about 3% (w/v) $Co(NO_3)_2 \cdot 6 H_20$ and about 20% (w/v) NH_4SCN.

7. The method according to any one of claims 1 to 6, wherein the sample further contains isooctylphenol polyoxyethylene ether (Triton™ X-100).

8. The method according to any one of claims 1 to 7, wherein the sample further contains tri-n-butyl phosphate (TNBP).

9. The method according to any one of claims 1 to 8, wherein alkaline hydrolysis in step (a) comprises hydrolysis with at least 3 N NaOH at a temperature of about 95°C to about 100°C for at least 45 minutes.

10. The method according to any one of claims 1 to 9, wherein the filter used in step (c) does not bind polyoxyethylene sorbitan.

11. The method according to any one of claims 1 to 10, wherein the non-water miscible organic solvent used in step (e) is selected from the group consisting of methylene chloride, chloroform, *o*-dichlorobenzene, bromoform, and trichloroethylene.

12. The method according to any one of claims 1 to 11, wherein the non-water miscible organic solvent is methylene chloride.

13. The method according to any one of claims 1 to 12, wherein the absorbance of the extract obtained in step (e) is measured in step (f) at about 324 nm.

14. The method according to any one of claims 1 to 13, further comprising after step (a) and before step (b) the step:

(a2) cooling the sample after alkaline hydrolysis.

15. The method according to any one of claims 1 to 14, further comprising after step (b) and before step (c) the step:

(b2) keeping the sample at room temperature for at least 30 minutes to allow the formation of denatured precipitate.