SUMMARY

Surprisingly, it was found that the disadvantages known from prior art may be overcome by performing a fluorescence micelle assay (FMA) using N-phenyl-1-naphthylamine (NPN) as fluorescence dye without a HPLC-system wherein polysorbate contained in a liquid sample is quantifiable in the presence of one or more peptides or polypeptides as well as proteins.

Therefore, a straightforward method to use a fluorescence micelle assay is provided. In one aspect, the present disclosure provides a method (a) for the quantification of polysorbate in an aqueous formulation containing polysorbate and one or more (poly)peptides having the following steps:

(a.1) providing a sample of the aqueous formulation;

(a.2) adding of a solution containing the fluorescent dye N-phenyl-1-naphthylamine to the sample of step (a.1) to obtain a fluorescent dye containing sample to be tested in a fluorescence micelle assay;

(a.3) optionally incubating the fluorescent dye containing sample of step (a.2);

(a.4) performing a fluorescence micelle assay with the fluorescent dye containing sample obtained in step (a.2) or step (a.3) and

(a.5) determining the content of polysorbate present in the sample from the fluorescence micelle assay performed in step (a.4);

with the proviso that the method (a) is being performed without the use of a HPLC-system.

Also a high-throughput quantitative analysis of polysorbate containing samples including one or more peptides or polypeptides as well as proteins is possible. Therefore, according to another aspect, it is provided a method (b) for the quantification of polysorbate in an aqueous formulation containing polysorbate and one or more (poly)peptides having the following steps:

(b.1) providing a number of samples of the aqueous formulation;

(b.2) adding of a solution containing the fluorescent dye N-phenyl-1-naphthylamine to each of the samples, respectively, to obtain fluorescent dye containing samples to be tested in a fluorescence micelle assay;

(b.3) optionally incubating the fluorescent dye containing samples of step (b.2);

(b.4) performing a fluorescence micelle assay with the fluorescent dye containing samples obtained in step (b.2) or step (b.3) and

(b.5) determining the content of polysorbate present in each of the samples from the fluorescence micelle assay performed in step (b.4);

with the proviso that the method (b) is being performed without the use of a HPLC-system.

It was unexpected that any kind of (poly)peptide, particularly protein, present in the starting aqueous formulation does not interfere with the measurement of the fluorescence micelle assay. Furthermore, it was

also unexpected that impurities, additives, excipients, do not interfere with the measurement of the fluorescence micelle assay.

Indeed, the methods of the present disclosure were found to reliably provide the content of polysorbate in aqueous formulations wherein at the same time one or more peptides, polypeptides, or proteins are present. In addition, the quantification can be employed for a series of test sets at the same time for a high-throughput quantitative analysis of polysorbate in samples containing peptide(s), polypeptide(s), or protein(s).

The disclosure is also directed to the use of the method according to the disclosure for the quantification of polysorbate in an aqueous formulation, for example, as a stability test of the aqueous formulation, during the development, manufacture, shelf life or storage time thereof, whereby the aqueous formulation contains polysorbate and one or more (poly)peptides as disclosed herein, with the proviso that the method is being performed without the use of a HPLC-system and the fluorescence micelle assay is performed in the presence of one or more peptides and/or one or more polypeptides, including one or more proteins.

In another aspect, the present disclosure provides use of the method as described herein, during the development, manufacture, shelf life, or storage time thereof, whereby the aqueous formulation contains polysorbate and one or more (poly)peptides.

In another aspect, the present disclosure provides use of a fluorescence micelle assay in a method for quantification of polysorbate in an aqueous formulation containing polysorbate and one or more (poly)peptides.

In another aspect, the present disclosure provides use of a fluorescence micelle assay in a method for quantification of polysorbate in an aqueous formulation containing polysorbate and one or more (poly)peptides as described herein.

A further subject of the present disclosure is also the use of a fluorescence micelle assay in a method for quantification of polysorbate in an aqueous formulation comprising polysorbate and one or more (poly)peptides as disclosed herein, with the proviso that the method is being performed without the use of a HPLC-system and the fluorescence micelle assay is performed in the presence of one or more peptides and/or one or more polypeptides, including one or more proteins.

BRIEF DESCRIPTION OF THE DRAWINGS

Embodiments of the prior art and of the present invention will be described by way of example with reference to the accompanying figures, which are schematic and are not intended to be drawn to scale so that no assumption of precise geometric values can be made regarding the original size. The figures of the present disclosure are incorporated in and constitute a part of the specification, also illustrating embodiments of the invention without limitation to the specific embodiments described. The drawings together with the general description and detailed description serve to explain the principles of the present disclosure. The same features are denoted by the same reference signs throughout the figures. It is shown:

Figures 1A and 1B	a schematic illustration of the concept of the fluorescence micelle assay;
Figures 2A and 2B	a schematic illustration of a sample containing polysorbate 20 (PS20) before and after adding the dye N-phenyl-1-naphthylamine used in the fluorescence micelle assay, whereby polysorbate 20 forms intact micelles;
Figures 3A and 3B	a schematic illustration of a sample containing polysorbate 20 (PS20) before and after adding the dye N-phenyl-1-naphthylamine used in the fluorescence