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Short communication

A simple reversed phase high-performance liquid chromatography method for polysorbate 80 quantitation in monoclonal antibody drug products

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ABSTRACT

In this paper, we discuss an improved high-performance liquid chromatography (HPLC) method for the quantitation of polysorbate 80 (polyoxyethylenesorbitan monooleate), a commonly used stabilizing excipient in therapeutic drug solutions. This method is performed by quantitation of oleic acid, a hydrolysis product of polysorbate 80. Using base hydrolysis, polysorbate 80 releases the oleic acid at a 1:1 molar ratio. The oleic acid can then be separated from other polysorbate 80 hydrolysis products and matrices using reversed phase HPLC. The oleic acid is monitored without derivatization using the absorbance at 195 nm. The method was validated and also shown to be accurate for the quantitation of polysorbate 80 in a high protein concentration monoclonal antibody drug product. For the measured polysorbate 80 concentrations, the repeatability was less than 6.2% relative standard deviation of the mean (% RSD) with the day-to-day intermediate precision being less than 8.2% RSD. The accuracy of the oleic acid quantitation averaged 94–109% in different IgG_1 and IgG_4 drug solutions with variable polysorbate 80 concentrations. In this study, polyoxyethylene, a by-product of the polysorbate 80 hydrolysis was also identified. This peak was not identified by previous methods and also increased proportionally to the polysorbate 80 concentration. We have developed and qualified a method which uses common equipment found in most laboratories and is usable over a range of monoclonal antibody subclasses and protein concentrations.

1. Introduction

Polysorbate 80 and other polysorbates are commonly used as stabilizing excipients in biopharmaceutical formulations [1]. In protein formulations, polysorbates minimize adsorption to surfaces, reduce the rate of protein denaturation and increase the drug solubility and stability [1,2]. Due to this critical role, accurate quantitation of polysorbate 80 is needed to assure product quality.

Polysorbate 80 is an oleate ester of sorbitol and its anhydrides copolymerized with 20 moles of ethylene oxide for each mole of sorbitol and sorbitol anhydride (Fig. 1). Due to its molecular heterogeneity and lack of a good chromophore, polysorbate 80 in its native form cannot be accurately analyzed by the conventional method of HPLC with UV absorbance detection [3]. Direct quantitation of polysorbate 80 by various other methods has been published in literature. Analysis of polysorbate 80 by high-performance liquid chromatography (HPLC) with evaporative light scattering detection (ELSD) was reported [3] but there was no mention of the drug protein concentrations used and the quantitation limit of the method was higher than the value reported here. Other methods also included (HPLC) with electrospray mass spectrometry (ESI) [4,5]. These methods analyzed polysorbate 80 in plasma samples and may not be suitable for analysis of polysorbate 80 in high protein concentration samples. Size exclusion chromatography with UV absorbance detection of polysorbate 80 has also been reported in the literature but showed a decrease in the polysorbate 80 quantitation limit when analyzed in the presence of protein [6]. Polysorbate 80 has also been analyzed by colorimetry but it required a multi-step sample preparation including solid-phase extraction and did not obtain the limit of quantitation reported in this work [7]. Although these methods allow for the direct quantitation of polysorbate 80 they could not obtain an equivalent quantitation limit as reported in this work or required extensive sample preparation, skilled analysts and/or specialized equipment.

Polysorbate 80 can be hydrolyzed at the ester linkage under basic conditions to release oleic acid at a 1:1 molar ratio. The oleic

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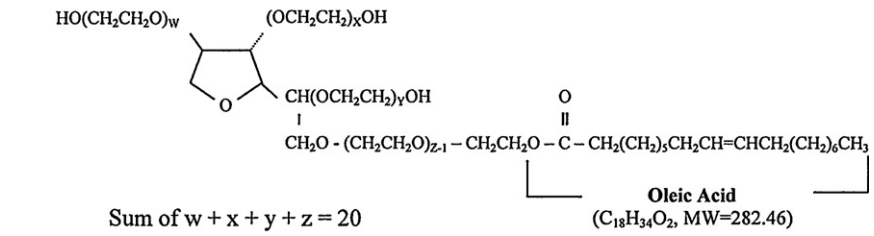
Polysorbate 80



Nonadecenoic Acid

$[CH_3(CH_2)_7CH{=}CH(CH_2)_8COOH]$ ($C_{19}H_{36}O_2$, MW = 296.49)

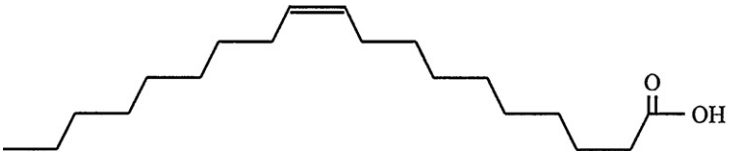


Fig. 1. Structures of polysorbate 80, nonadecenoic acid and oleic acid.

acid can then be analyzed by standard RP-HPLC methods with detection by UV absorbance. Using the free fatty acid from hydrolysis of polysorbates for their quantitation has been previously reported [8–10]. The methods which allowed for the quantitation of polysorbate 80 were either investigated primarily in the pharmaceutical suspension without protein [9] or with protein concentrations less than 35 mg/mL [10]. This paper describes an improved RP-HPLC method using alkali hydrolysis and standard UV detection for the quantitation of polysorbate 80 in the presence of high protein concentration samples (up to 75 mg/mL). This method incorporates an extraction step which can increase the usable life of the column since sodium hydroxide and any unhydrolyzed proteins from the high protein concentration samples will be not be injected on the system. This method is also able to separate oleic acid from polyoxyethylene. None of the other methods identified this polyoxyethylene peak which also increased proportionally to the polysorbate 80 concentration. The peak identities of oleic acid and polyoxyethylene were confirmed by mass spectrometry analysis. This method was validated and used successfully for the quantitation of polysorbate 80 down to 20 ppm in a range of monoclonal antibody subclasses and at protein concentrations up to 75 mg/mL.

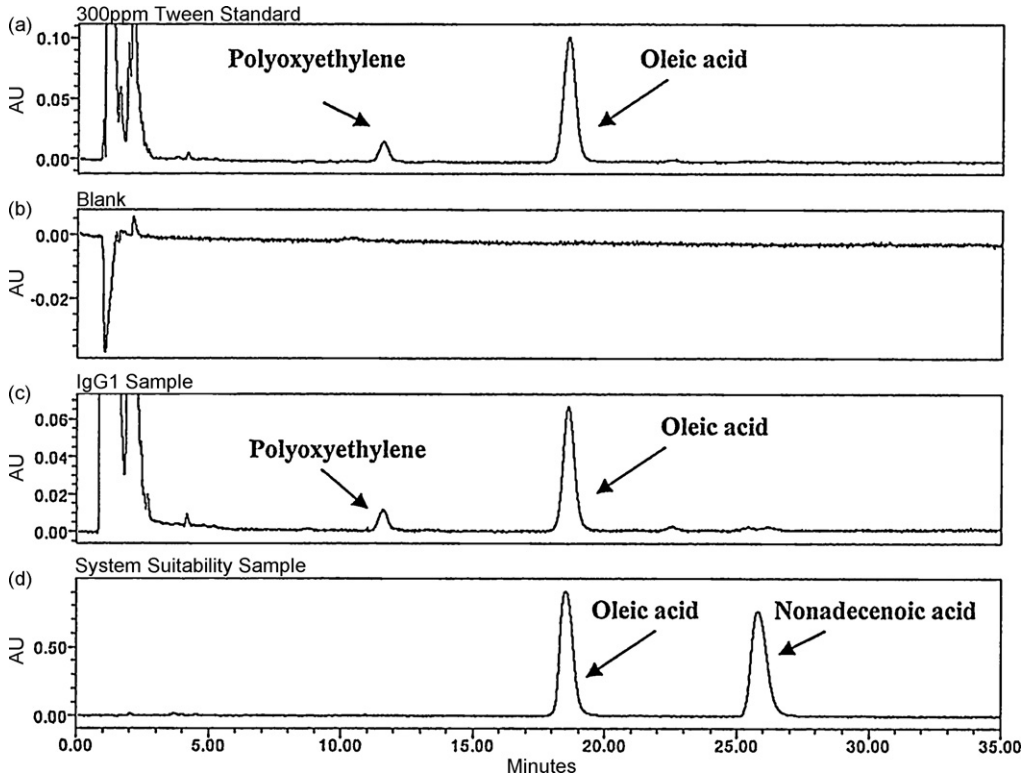


Fig. 2. A hydrolyzed polysorbate 80 sample compared to oleic acid, nonadecenoic acid and a formulation blank chromatogram. (a) Polysorbate 80 standard; (b) formulation buffer blank; (c) IgG_1 formulated with polysorbate 80; and (d) system suitability sample containing oleic and nonadecenoic acids.