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Original article

Development of a simple high performance liquid chromatography (HPLC)/evaporative light scattering detector (ELSD) method to determine Polysorbate 80 in a pharmaceutical formulation

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ABSTRACT

The amount of polysorbate 80 in pharmaceutical formulations affects the product quality and efficacy. A reliable test method is required to quantify the amount of Polysorbate 80 present in the drug product formulations. The test method for the determination of Polysorbate 80 may be used during process development and final product quality assessment. A simple, fast and efficient quantitative method, making use of HPLC-ELSD and a C18 column without sample pretreatment was developed. The developed method demonstrated specificity to polysorbate 80 with high precision as indicated by percent relative standard deviation (%RSD) of 3.0% for six determinations. The accuracy of this method for the determination of polysorbate 80 in a pharmaceutical formulation was demonstrated with an overall recovery of 94.9%.

1. Introduction

Polysorbate 80, also known as Tween 80 is used as a non-ionic emulsifier in this drug product, amounts of Polysorbate 80 affects product quality and emulsion stability. Polysorbate 80 consists of a sorbitol moiety with 20 units of polyoxyethylene (—CH_2CH_2O—) group and one oleic acid group attached as shown in Fig. 1. A Polysorbate 80 acts as a vehicle that enhance the solubility of active pharmaceutical ingredient (API) in water. There is a need to quantify the amount of Polysorbate 80 present in the drug product formulations during process development and final product quality assessment. This method was developed to address these requirements.

Commercially available Polysorbate 80 is a chemically diverse mixture containing mainly sorbitan polyoxyethylene (POE) fatty acid esters. Substantial amounts of POE, sorbitan POE and isosorbide POE fatty acid esters can also be present. The heterogeneous nature of the Polysorbate 80 makes it difficult to quantify using a conventional method. Several quantitative methods have been developed and published in the literature to quantify Polysorbate 80 and to determine its composition (Tani et al., 1997, Hewitt et al., 2011, Adamo et al., 2010, Christiansen et al., 2011, Nayak et al., 2012, Nair et al., 2003, Hu et al., 2003). Since Polysorbate 80 does not have sufficient chromophore to absorb UV radiation, UV based high-performance liquid chromatography methods are unsuitable. The analytical methods based on hydrolyzing the oleic acid and then quantifying using UV method were developed (Adamo et al., 2010, and Hu et al., 2003). One disadvantage of the acid hydrolyzing pretreatment method is its lack of selectivity/specificity. Due to the presence of castor oil and oleic acid in the drug product a hydrolyzing method is unsuitable for this application.

Several HPLC/UPLC-CAD/MS methods showed multiple peaks from Polysorbate 80 during its chromatographic separation due to its complex molecular structures (Pan et al., 2016; Zhang et al., 2012). Efforts were made to elute Polysorbate 80 as a single peak and then quantify it using ELSD detector. Consequently this method required a chromatographic system washing with 100% methanol for 60 min between injections (Nair et al., 2003). Size exclusion chromatography (SEC) technique coupled with a UV detector was also explored to quantify the Polysorbate 80 in different formulations, but details such as specificity, accuracy and precision data are unavailable (Klein and Preston). Also chromatograms provided in the literature showed multiple peaks. Nayak et al., (2012) developed a method based on

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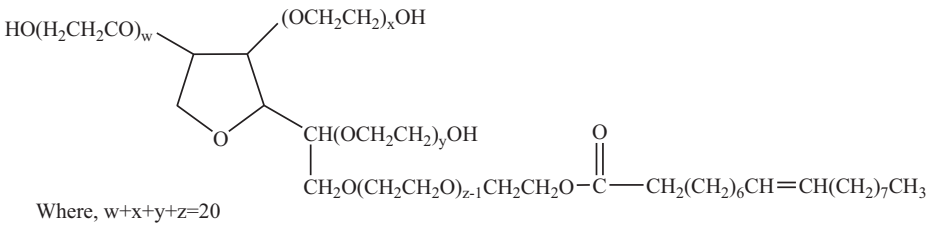

Fig. 1. Structure of Polysorbate 80 (Tween 80).

high-performance liquid chromatography (HPLC) coupled with an evaporative light scattering detection (ELSD) to quantify Polysorbate 80 in protein formulations. This method consists of removal of protein by solid phase extraction followed by chromatographic analysis. The absence of multiple peaks, in this case, may be due to the elimination of other late-eluting Polysorbate 80 components during the extraction process, or the solvents strength of the gradient was not strong enough to wash out late-eluting components present in Polysorbate 80.

To keep our method simple, fast and efficient, the use of HPLC-ELSD and a C18 column was explored. Preliminary experiments were focused on using acetonitrile and water in gradient conditions to elute the Polysorbate 80 peak, as observed in the literature, without utilizing any solid phase extraction or initial sample preparations (e.g., derivatization or hydrolysis). Tetrahydrofuran (THF) was introduced at a later stage of the chromatographic gradient to remove late-eluting peaks from Polysorbate 80 and other components (or excipients) present in the ophthalmic emulsion thus achieving reproducible chromatography. Based on the polysorbate 80 standard and drug product chromatograms, peak eluting around 8.5 min was used for quantitation of Polysorbate 80 in this drug product. The specificity, precision, and accuracy of the method were studied to evaluate method performance.

2. Materials and methods

2.1. Materials

Polysorbate 80 was purchased from Corda, Inc, NJ, USA. Methanol and Tetrahydrofuran were HPLC grade and purchased from Fisher Scientific, NJ, USA. An ophthalmic emulsion (drug product) formulated in the laboratory was used for analysis. The drug product contained several inactive components including Polysorbate 80, Carbomer copolymer, glycerin, castor oil, sodium hydroxide and water for injection in addition to active pharmaceutical Ingredient (API).

2.2. Chromatographic system and chromatographic parameters

The high-performance liquid chromatography (HPLC) system consists of an Agilent 1200 Series HPLC system (Agilent Technologies, Palo Alto, CA, USA) equipped with a Quaternary pump and auto-injector coupled to a 1260 Infinity (Agilent Technologies, Palo Alto, CA, USA) evaporated light scattering detector (ELSD). In house nitrogen supplies from Titus nitrogen generator (PA, USA) was used as the source of the nitrogen gas for ELSD. An HPLC column Gemini C18, 150 × 4.6 mm, 3 μm (Phenomenex, Torrance, CA, USA) packed with C18 stationary phase with Triethylamine Silane (TMS) end-capping was used. The mobile phase A, B, C consisted of acetonitrile, water, and tetrahydrofuran respectively. The gradient conditions were as follows (see Table 1).

The column temperature was maintained at 40 °C, and a flow rate of 1.0 mL/min. The injection volume was 50 μL. ELSD was operated at drift tube temperature of 80 °C, a gain setting of 10, and nitrogen pressure at 50 psi. Data were collected and analyzed using Chromeleon 7.2 chromatography Data system (Thermo Fisher Scientific Inc, MA, USA).

Table 1
Gradient table.

Time (min)	A (%)	B (%)	C (%)
0.0	5.0	95.0	0.0
5.0	5.0	95.0	0.0
5.0	60.0	40.0	0.0
10.0	80.0	20.0	0.0
12.0	50.0	10.0	40.0
22.0	50.0	10.0	40.0
22.1	5.0	95.0	0.0
25.0	5.0	95.0	0.0

2.3. Test samples

An Ophthalmic emulsion was formulated with an Active Pharmaceutical Ingredient (API), and inactive excipients which include; Castor Oil, Glycerin, Carbomer Copolymer Type A, and Polysorbate 80. Methanol was used as a diluent for all samples preparation.

Specificity samples were prepared using standard materials of Polysorbate 80, API, Castor oil, Carbomer Copolymer Type A (Pemulen TR-2), and Glycerin.

A Quadratic 2nd order calibration curve with concentration levels of 0.20, 0.30, 0.40, 0.50, 0.60 and 0.80 mg/mL was used for quantifying the Polysorbate 80.

Precision samples were prepared by adding accurately around 100 mg of drug product in 10 mL volumetric flask and then an accurate amount of Polysorbate 80 stock standard solution was added to achieve a concentration of 0.5 mg/mL Polysorbate 80. Finally, the samples were brought to volume with methanol (diluent). Six samples were prepared for the precision study.

To demonstrate accuracy, drug product was spiked with Polysorbate 80 standard solution at three different levels (40%, 100%, and 140%) of the nominal sample concentration of 0.50 mg/mL. The accuracy samples were prepared as follows; accurately about 100 mg of drug product was added to a 10 mL volumetric flask an accurate amount of Polysorbate 80 stock standard solution was added to achieve the required concentration level and the samples were brought up to volume with methanol. Accuracy samples were prepared in triplicate at each level and a non-spiked sample was prepared as a control in duplicate. The percentage recovery was calculated at each spiked level after adjusting for the Polysorbate 80 contribution from the drug product.

3. Results and discussion

3.1. Initial method development and specificity of the method

The primary objective of this study was to develop a simple HPLC method to quantify Polysorbate 80 (PS80) in an ophthalmic emulsion, which contains Castor Oil, Glycerin, Carbomer Copolymer Type A, and Polysorbate 80. An initial HPLC method using a

C18 column with acetonitrile and water as eluent was used to achieve better peak shape and separation between various components present in the drug product. Tetrahydrofuran (THF) as an eluent was then introduced in the later stage of the gradient to clean the column and the overall chromatographic system. The introduction of THF resulted in reproducible chromatograms after several sample injections containing castor oil. Also, our initial method development effort was to develop a method without any sample pretreatment such as solid phase extraction (SPE) step or derivatization. Previous studies indicated the presence of multiple peaks from PS80 sample (Pan et al., 2016; Zhang et al., 2012). Multiple peaks of Polysorbate standard as shown in Fig. 2 below were observed. The late eluting peaks which elute after 14 min are from the hydrophobic component of PS80 and the peak around 8.5 min (see Fig. 2) was chosen as the peak of interest and used for quantitation of Polysorbate 80 in this study.

The specificity of the method was evaluated by injecting a standard solution of API, castor oil, and Carbomer Copolymer Type A, and Glycerin. The representative chromatograms can be seen in Fig. S1 (Supporting Information). No interference to the Polysorbate 80 peak at RT ~ 8.5 min was observed. The API peak (RT ~ 14.2 min) co-eluted with the late eluting peaks from PS80 (See Fig. S1, Supporting Information). One of the late-eluting Polysorbate 80 peak [from retention time (RT) of 14.1–15.9 min] which was not considered for quantification, co-eluted with the API peak of this drug product (see Fig. S1, Supporting Information). In summary, the method is specific for the determination of Polysorbate 80 in this pharmaceutical formulation.

3.2. Calibration curve

Because the ELSD response increases with an increase in Polysorbate 80 concentration in a non-linear manner (Nayak et al., 2012), a second order quadratic standard curve was used to quantify the Polysorbate 80 present in the drug product. Six Polysorbate 80 standard solutions in the concentration range of 0.2 mg/mL to 0.8 mg/mL were injected to obtain a second-order quadratic calibration curve with an R-squared value of 0.9998 (see Fig. S2, Supporting Information). An R-squared value of 0.9998 suggests an acceptable goodness-of-fit of the detector response to the second order quadratic model.

3.3. Precision

The repeatability of the method was evaluated by injecting six samples prepared by spiking standard Polysorbate 80 solution at the concentration level of 0.5 mg/mL (See Fig. S3, Supporting Information; for a representative chromatogram). The average percent recovery, standard deviation, and percent relative standard deviation were calculated. The average percent recovery for the six samples was 96.0%, and the relative standard deviation was 3.0%. This low percent relative standard deviation indicated the method is precise for determination of Polysorbate 80.

3.4. Accuracy

To evaluate the accuracy of the method, the percent recovery of Polysorbate 80 from the spiked samples prepared at three different concentration levels i.e., at 40, 100 and 140% of the nominal concentration of 0.5 mg/mL was determined. Three individual sample preparations at each level were analyzed (See Figs. S4 to S6, Supporting Information; for representative chromatogram at each concentration level). The average percent recovery at each level and the percent relative standard deviation (%RSD) was calculated (see Table 2). The recovery was 93.0% (%RSD = 6.0, n = 3, at 40% level), 96.8% (%RSD = 2.6, n = 3, at 100% level) and 95.2%

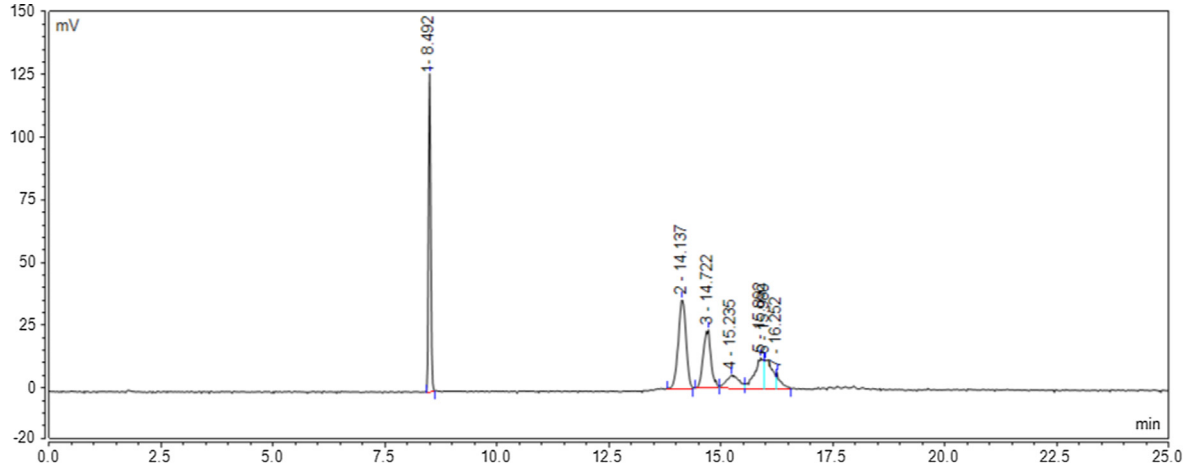

Fig. 2. Representative chromatogram of a Polysorbate 80 standard solution (0.5 mg/mL in methanol).

Table 2
Accuracy data from recovery study at three different levels.

Accuracy level	% Recovery	%Recovery (Average)	Standard Deviation	%RSD	% Recovery (over all)	
					Average	%RSD
40%-Level	99.4 88.9 90.7	93.0	5.6	6.0	94.9	3.7
100%-Level	98.0 94.2 93.3	96.8	2.5	2.6		
140%-Level	96.9 97.7 95.7	95.2	1.0	1.0		

(%RSD = 1.0, n = 3, at 140% level). The overall % recovery and % RSD from all determinations (n = 9) was 94.9 and 3.7 respectively. The above data indicated that our method accurately determined the amount of Polysorbate 80 present in the ophthalmic emulsion.

4. Conclusion

An HPLC method using ELSD detector was developed for the determination of Polysorbate 80 in a pharmaceutical formulation. This method can determine the amount of Polysorbate 80 present in the drug product without any sample pretreatment. The developed method is simple, reproducible, accurate and precise for its purpose. As mentioned earlier multiple peaks have been observed in reverse phase chromatography due to the heterogeneous molecular structure of Polysorbate 80. The accurate quantitation of Polysorbate 80 using this method would depend on the selection of Polysorbate 80 standard as only one peak was used for quantification purpose. The composition of Polysorbate 80 and batch-to-batch variations (even from the same manufacturer) in the composition of Polysorbate 80 are commonly observed, hence it is preferred to use the same lot of Polysorbate 80 as a standard which was used for the formulation of the drug product. The method performance also depends on the assumption that during the formulation and storage of the drug product that there is no change in the composition of Polysorbate 80 (particularly the component which elutes at RT ~ 8.5 min) if same lot polysorbate 80 used as standard. Care should be given to the detector's cleanliness when performing the analysis to achieve consistency in the results. The method described here can be used to determine the Polysorbate 80 in a pharmaceutical formulation. Future study has been planned to validate the method as per ICH guidelines.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jsps.2020.01.012.

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