Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1012 (2003) 81–86
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Determination of polysorbate 80 in parenteral formulations by high-performance liquid chromatography and evaporative light scattering detection

Lakshmy M. Nair*, Norma V. Stephens, Sarah Vincent, Neervalur Raghavan, Patrick J. Sand

Medication Delivery, Baxter Healthcare, Round Lake, IL 60073, USA

Received 23 January 2003; received in revised form 16 June 2003; accepted 17 June 2003

Abstract

Drugs that are not very soluble in aqueous formulations are solubilized with surfactants such as polysorbate 80. In order to evaluate the stability of excipient such as polysorbate 80 in drug formulation, a rapid chromatographic methodology is desired; however, polysorbate 80 does not have a strong chromophore for monitoring by absorption spectrometry. A simple and fast method for the analysis of polysorbate 80 in pharmaceutical formulations was developed using high-performance liquid chromatography with evaporative light scattering detection (ELSD). Separation of polysorbate 80 as a single peak was achieved on a C18 column using a methanol/water gradient mobile phase and ELS detection. The method is specific for polysorbate 80 in the formulation as there were no interferences from the drug or other excipients. Precision, recovery, linearity and limit of quantitation/detection experiments gave acceptable results during the evaluation of the method.

Keywords: Pharmaceutical analysis; Polysorbate

1. Introduction

Surfactants are widely used in the pharmaceutical industry as solubilizers for insoluble drugs. When surfactants are added to water above the critical micelle concentration, the solubility of hydrophobic drug compounds greatly increases. An example surfactant that is commonly used for this purpose is polysorbate 80 (commercially known as Tween 80) or polyoxyethylene (20) sorbitan mono-oleate (Fig. 1). Polysorbate 80 is a non-ionic surfactant possessing high surface activity and low toxicity, which makes it a good candidate for pharmaceutical applications.

A method to monitor the stability of polysorbate 80 in a drug formulation was necessary as the drug solubility depended on the polysorbate 80 concentration. It is also common in the pharmaceutical industry to quantitate each component in the drug formulations during various stages of drug formulation development. For these reasons, it was necessary to develop a method to quantitate polysorbate 80 in the formulation.

Polysorbates have heterogeneous molecular struc-

*Corresponding author. Tel.: +1-847-270-5942; fax: +1-847-270-5999.
E-mail address: lakshmy_nair@baxter.com (L.M. Nair).

0021-9673/03/$ – see front matter
doi:10.1016/S0021-9673(03)01105-1

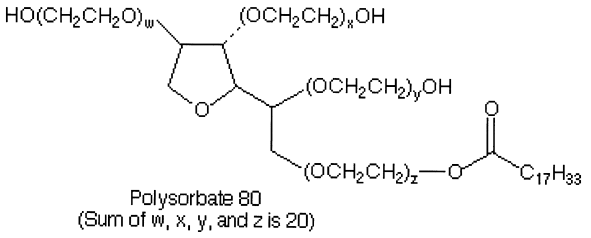

Fig. 1. Structure of polysorbate 80.

tures, hence they pose many challenges to their analytical determination. Several attempts to quantitate polysorbate 80 have been reported in the literature. Some of the methods used are based on colorimetry, thin layer chromatography (TLC), and high-performance liquid chromatography (HPLC) [1–3,5]. All of these published chromatographic methods suffered from inadequate separation and resolution. In another paper, polyoxyethylene-type emulsifiers were analyzed using matrix assisted laser desorption/ionization time-of flight mass spectrometry (MALDI-TOF–MS) [4]. This method suffered from producing poor quality spectra and lack of identification of all peaks. HPLC with UV detection is the most common analytical technique used for the analysis of surfactants, but lack of sufficient chromophore in polysorbate 80 made it difficult to analyze it by these conventional HPLC methods. Hydrolysis of polysorbate 80 to release oleic acid by treating with a base or acid while heating, followed by determination of oleic acid has also been reported [6]. Such methods are laborious and time consuming and therefore inappropriate when large sample throughput is required.

In this paper we report a simple and fast method for the determination of polysorbate 80 using reversed-phase HPLC separation with evaporative light scattering detection (ELSD). ELSD, known as the mass detector has become popular as a universal detector in the recent past [7,8]. ELSD measures the light scattered by droplets or particles of non-volatile analyte compounds after volatile compounds of mobile phase have been evaporated. Hence, the detection characteristics are not dependant on the optical characteristics of the analyte. Concentrations of analytes are directly proportional to the amount of light scattered.

Applications of ELSD for the determination of surfactants, polymers, carbohydrates and triglycerides are widely reported [8–11]. In the pharmaceutical industry, ELSD is used for the determination of drugs, impurities, raw materials and inorganic counter-ions [12–15]. ELSD is suitable for polysorbate 80 determinations since it has a weak UV chromophore but has non-volatile characteristics. An HPLC–ELSD method for polysorbate 80 [16] determination was reported earlier, however, the analyte was separated into multiple component peaks. Such a multi-peak chromatogram is not ideal for quantitation, so a gradient system is developed in this paper for producing a single chromatographic peak. This method was successfully used for determining polysorbate 80 concentrations during the formulation stability studies.

2. Experimental

2.1. Chromatographic system

The HPLC system consisted of a Waters 2695 HPLC system (Waters, Milfred, MA, USA) coupled with an Alltech ELSD 2000 Detector (Alltech Associates, Deerfield, IL, USA). An ELSD nitrogen generator (Alltech Associates) was used as the source for the nitrogen gas. A HPLC column (250×4.6 mm) packed with Alltima C18, 5-μm (Alltech Associates) was used to separate polysorbate 80 from other peaks. ELSD was operated in the Impactor ‘‘On’’ mode and the drift tube temperature was set at 40 °C. Nitrogen flow was maintained at 1.5 l/min. The theory and operation of Alltech ELSD 2000 has been discussed in the literature [17].

2.2. Reagents

Methanol (HPLC grade) was purchased from Burdick and Jackson Labs (Muskegon, MI, USA). Distilled water from a source available in the laboratory was used throughout the analyses. Nitrogen gas (ultra-pure, >99%) was produced using the nitrogen generator (Alltech Associates). Polysorbate 80 raw material was purchased from Croda (Parsippany, NJ,

USA). Both standards and samples were prepared from the same lot of raw material. Standards were prepared by dissolving polysorbate 80 in distilled water. Formulation samples were injected directly without any further sample preparation.

2.3. Gradient conditions

The mobile phase consisted of methanol (A) and water (B). The 12-min gradient program was increased from 30% to 90% methanol from initial to 4 min at a flow-rate of 1.0 ml/min. Complete gradient conditions are as follows:

Time (min)	A (%)	B (%)
0.0	30	70
4.0	90	10
12.0	30	70

2.4. Test samples

Test samples in this study were prepared from commercially available raw materials and pharmaceutical products. A typical formulation sample contained drug, polysorbate 80, dextrose, water for injection, and hydrochloric acid or sodium hydroxide for pH adjustment. Stock solutions of dextrose, polysorbate 80, and drug/polysorbate 80 were prepared and they were further diluted to prepare test articles (TAs), control and matrix solutions. Three different solutions with pH values of 3.1, 3.5 and 2.5 were prepared at 100% level (4 mg/ml polysorbate 80). To maximize potential interference, levels of drug and dextrose were prepared at about 110% of the nominal formulation concentrations.

Additionally, thermally stressed samples were prepared and analyzed for possible heat degradation products and their interference with polysorbate 80. These samples included three different solutions: a 50 mg/ml dextrose solution at a pH value of 3.1, a 4.0 mg/ml polysorbate 80 solution at a pH value of 3.1, and a proposed formulation containing dextrose, polysorbate 80, and the drug at a pH value of 3.1. These solutions were filled into plastic and glass container systems, and heat stressed in a chamber at 70 °C for 48 h.

3. Results and discussion

3.1. Separation of polysorbate 80 as a single peak

The scope of this study was to develop a method for the separation of polysorbate 80 as a single peak for the quantitation of polysorbate 80 in multiple formulation samples. A highly non-polar C18 column (25×4.6 mm) was chosen for the separation of polysorbate 80 due to its hydrophilic characteristics. Several gradient compositions were evaluated to obtain a single polysorbate 80 peak. Optimized gradient conditions are described in the Experimental section. The gradient starts with a water/methanol ratio of 70:30 and at 4 min changes to water/methanol (10:90). This sudden increase in the strength of the mobile phase was necessary for minimizing multiple peaks and obtaining a single peak. An example chromatogram of polysorbate 80 (4 mg/ml) standard solution prepared in water is shown in Fig. 2. A Gaussian peak was not obtained because the single peak is formed by compressing multiple distribution peaks caused by the heterogeneous molecular species of polysorbate 80. The peak shape was adequate for our purpose since our intention was not to resolve or quantitate individual polymer species, but to quantitate total polysorbate 80 in the formulation samples.

3.2. Analysis of polysorbate 80 in parenteral formulation

The proposed analytical method was evaluated for the determination of polysorbate 80 in one of the Baxter injectable formulations. The parameters evaluated were specificity, accuracy, precision and linearity, limit of quantitation and limit of detection.

3.2.1. Specificity

Specificity was assessed by injecting samples of water, matrix and proposed formulation, as well as solutions of polysorbate 80 (in both glass and plastic containers) that were subjected to sufficient thermal stress (70 °C for a minimum of 48 h) to evaluate possible interferences due to container extractives. All thermally stressed solutions prepared in plastic containers were compared to the same solutions in glass under identical conditions to facilitate the

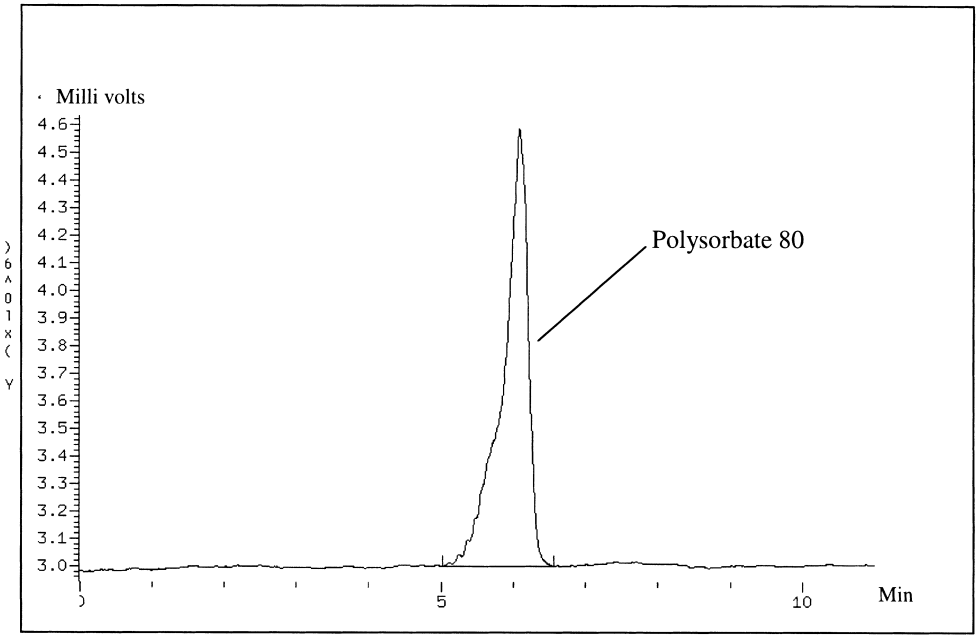

Fig. 2. Polysorbate 80 standard (4.0 mg/ml) chromatogram.

identification of potential container related peaks. No interferences from drug or dextrose were noted in any of the specificity samples. Thermally stressed samples also presented no evidence of interference due to container extractives.

3.2.2. Accuracy and precision

Accuracy, precision and linearity were evaluated by analyzing test solutions prepared at approximately 80%, 100% and 120% levels of polysorbate 80 concentrations in the formulation. Results for accuracy, precision and linearity are reported in Tables 1 and 2. Sample recovery ranged from 97.52 to 102.11%, with %RSD values ranging from 0.2 to 1.3%. An example chromatogram of sample containing approximately 4 mg/ml of polysorbate 80 is shown in Fig. 3.

Carryover was a major issue to deal with during this assay development. A methanol injection and column flush (100% methanol for 60 min) was performed periodically during the course of the HPLC run to reduce material buildup in the column. This clean-up procedure was helpful to maintain the system precision throughout the HPLC analysis. The

Table 1
Accuracy and precision

Test solution	Theoretical conc.[a] (mg/ml)	Mean experimental conc. (mg/ml polysorbate 80)	Mean recovery (%)	95% confidence interval	RSD (%)
Matrix	–	–	–	–	–
Control	4.05	4.14	102.10	1.9	0.8
TA-80-3.1	3.10	3.09	99.53	1.2	0.5
TA-100-2.5	4.00	3.94	98.55	3.2	1.3
TA-100-3.1	4.00	3.93	98.36	3.2	1.3
TA-100-3.5	4.00	3.90	97.52	0.6	0.2
TA-120-3.1	4.90	4.93	100.60	1.9	0.8

TA, test article.
[a] Theoretical values for test solutions are the mean experimental concentrations obtained for the control corrected for the dilution.

Table 2
Linearity

Calculation	Standard range 50–150%	Samples range 80–120%
Slope (mV/s per mg)	1.37×10^{7}	1.39×10^{7}
y-intercept (mV/s)	-1.45×10^{6}	-1.5×10^{6}
% *y*-intercept[b]	−26.4	−27.5
Correlation coefficient (r^2)	0.997	0.996
Residual sum of squares	1.64×10^{13}	4.04×10^{12}

[a] Computed versus the mean response of test articles at 100% level.

overall system precision was calculated to be 1.9% RSD.

3.2.3. Linearity

Standard linearity was evaluated by triplicate injections of standard solutions prepared from polysorbate 80 solutions of concentrations ranging from 2 to 6 mg/ml and resulted in a correlation coefficient of 0.997. Sample linearity data were obtained from the accuracy study. The correlation coefficient for the sample was calculated to be 0.996. Table 2 shows the results from the standard and sample linearity study. ELS has the drawback a non-linear response and one must use calibration curves to address this issue [8]. A three-point calibration curve was used (2.0, 4.0, 6.0 mg/ml) for the quantitation of polysorbate 80 in the formulation samples.

3.2.4. Limit of quantitation (LOQ) and limit of detection (LOD)

The LOQ for polysorbate 80 in the formulation was estimated to be approximately 40 μg/ml based on a signal-to-noise ratio of 10. The LOD for polysorbate 80 by this method was determined to be approximately 15 μg/ml (S/N~3). Both the LOQ and LOD were determined by serially diluting sample preparations containing the lowest level of polysorbate 80.

4. Conclusion

An HPLC method using ELSD was evaluated for the total quantitation of polysorbate 80 in one of the Baxter parenteral formulations. The ability to quantitate polysorbate 80 as a single peak is clearly an advantage of this method over other existing methods. Simplicity and low maintenance of ELSD system are other useful features that were helpful in monitoring polysorbate 80 concentrations in multiple

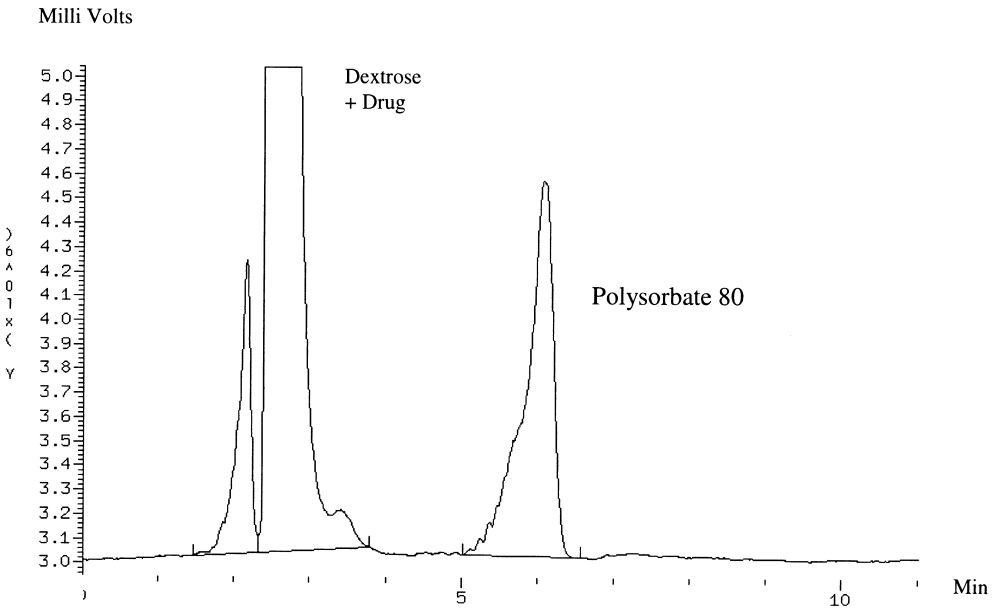

Fig. 3. Polysorbate 80 in parenteral formulation.

formulation samples during our formulation studies. Method precision, accuracy and linearity experiments gave acceptable results in the ranges evaluated.

References

[1] D.H. Daniels, C.R. Warner, S. Selim, J. Assoc. Off. Anal. Chem. 65 (1982) 162.
[2] H. Kato, Y. Nagai, K. Yammamoto, Y. Sakabe, J. Assoc. Off. Anal. Chem. 72 (1989) 27.
[3] J.M. Murphy, C.C. Scott, Analyst 94 (1969) 481.
[4] G.A. Cumme, E. Blume, R. Bublit, H. Hoppe, A. Horn, J. Chromatogr. A 791 (1997) 254.
[5] T.H. Tani, J.M. Moore, T.W. Patapoff, J. Chromatogr. A 786 (1997) 99.
[6] Z. Oszi, G. Petho, J. Pharma. Biomed. Anal. 18 (1998) 715.
[7] G.R. Bear, J. Chromatogr. 459 (1988) 91.
[8] Y. Mengernik, H.C.J. De Man, S.J. Van Der Wal, J. Chromatogr. 552 (1991) 593.
[9] B.L. Neff, H.J. Spinelli, J. Appl. Polym. Sci. 42 (1991) 595.
[10] R. Macrae, J. Dick, J. Chromatogr. 210 (1981) 138.
[11] W.S. Lettar, J. Liquid Chromatogr. 16 (1) (1993) 225.
[12] J.G. Wilkes, J.B. Sutherlans, M.I. Churchwell, A.J. Williams, J. Chromatogr. 695 (1995) 319.
[13] M.D. Lantz, D.S. Risley, J.A. Peterson, J. Liquid Chromatogr. Relat. Technol 20 (1997) 1409.
[14] D.S. Risley, J.A. Peterson, K.L. Griffiths, S. McCarthy, LC GC 14 (1995) 1040.
[15] D.S. Risley, K.F. Hostettler, J.A. Peterson, LC GC 16 (1998) 562.
[16] Alltech Associates, ELSD 2000 Product Literature, Bulletin # 424, Deerfield, IL, 2000.
[17] A. Kuch, R. Saari-Nordhaus, Am. Lab. March (2001) 61.