Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 984 (2003) 233–236
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

High-performance liquid chromatographic determination of polysorbate 80 in pharmaceutical suspensions

M. Hu, M. Niculescu, X.M. Zhang*, A. Hui
Novex Pharma, 380 Elgin Mills Road East, Richmond Hill, Ontario, Canada L4C 5H2

Received 23 July 2002; received in revised form 7 November 2002; accepted 8 November 2002

Abstract

A quick HPLC method is reported for the analysis of polysorbate 80 in pharmaceutical suspensions. A typical pharmaceutical suspension was mixed with dilute potassium hydroxide, and heated at 40 °C for 6 h. This procedure resulted in quantitative hydrolysis of polysorbate 80 to release oleic acid. A quick HPLC procedure was used to analyze the hydrolyzed samples without further sample treatment. Polysorbate 80 USP, treated in the same way as the pharmaceutical suspensions, was used as standard. Full validation tests were carried out and the validation studies demonstrated that this method is suitable for accurate and reproducible analysis of polysorbate 80 in pharmaceutical suspensions.

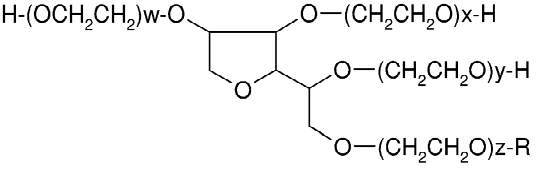
Keywords: Polysorbate 80

1. Introduction

Polysorbate 80 as a nonionic surfactant, is widely used in liquid pharmaceutical products such as inhalation suspensions and nasal suspensions as a result of its properties of solubilization, reduction of surface and interfacial tension and wetting [1]. For example, in the liquid formulation of protein therapeutics, surfactants are added to minimize protein absorption to surfaces (containers and syringe) and to reduce the air–liquid interfacial surface tension in order to decrease the rate of protein denaturation that can lead to aggregation [2–4].

The polysorbates are synthesized by copolymerizing sorbatan anhydride and 20 mol of ethylene oxide. A fatty acid is esterified to one of the terminal hydroxyl groups of the polyoxyethyleneoxide side chains. The type of attached fatty acid molecule is reflected in the numbers in the name of polysorbates. In case of the polysorbate 80, the fatty acid is oleic acid ($C_{17}H_{33}COOH$). The structure is shown in Fig. 1.

The existing methods for polysorbate 80 analysis



w + x + y + z = 20, and R = $CH_3(CH_2)_7CH{=}CH(CH_2)_7CO$

Fig. 1. Structure of polysorbate 80.

*Corresponding author. Tel.: +1-905-884-2050; fax: +1-905-884-9876.
E-mail address: xzhang@novexpharma.com (X.M. Zhang).

0021-9673/02/$ – see front matter
PII: S0021-9673(02)01849-6

are quite time consuming and use environmentally hazardous solvents [5–9]. Briefly, a suitable volatile organic solvent was used to extract polysorbate 80 from the sample mixture. After evaporating the organic solvent, the residual was re-dissolved with a mixture of aqueous cobalt and methylene chloride. Polysorbate 80 formed a complex with cobalt and then partitioned to the organic phase. The concentration of polysorbate 80 in the organic phase was then quantified by optical absorption. Tani et al. described a single-step HPLC method to quantify polysorbate 80 [10]. This method used size-exclusion chromatography (SEC) and a mobile phase containing surfactant at concentrations above the critical micelle concentration. Polysorbate 80 appeared as a broad peak, even through the presence of micellar surfactant in the mobile phase helped to shorten the elution time of the surfactant and disrupt any surfactant–column interactions.

Fatty acids can be easily determined by high-performance chromatography (HPLC) [11–15]. Hydrolysis of polysorbate 80 releases oleic acid. Some hydrolysis procedures for quantitative release of fatty acids in polymers or biomolecules have been reported [16–20]. Thus, if a suitable hydrolysis procedure could be established to quantitatively release oleic acid, RP chromatography could be used to determine the amount of oleic acid, and then polysorbate 80. In this paper we report a quick RP-HPLC method for the quantitative determination of polysorbate 80. This procedure was based on the hydrolysis of polysorbate 80 to release oleic acid followed by RP-HPLC separation and UV detection of oleic acid without further sample treatment. Since polysorbate 80 was used as standard and treated in the same way as the samples, this chromatographic analysis directly gave the analytical results of polysorbate 80 in pharmaceutical suspensions.

2. Material and methods

2.1. Hydrolysis of polysorbate 80

Thoroughly mix 9.0 ml of a typical pharmaceutical suspension and 1.0 ml of 1 *M* KOH solution in a flask. Heat this mixture in a 40 °C water bath for 6 h. Cool the mixture to room temperature and filter a portion through a 0.45-μm nylon filter into a HPLC vial.

2.2. HPLC methodology

2.2.1. Mobile phase preparation

The phosphate solution was prepared by dissolving 2.76 g of potassium phosphate, monobasic in 1000 ml of purified water. This mobile phase was composed of acetonitrile and the buffer solution (8:2, v/v) and pH was adjusted to 2.8±0.1 with 85% phosphoric acid.

2.2.2. Sample preparation

2.2.2.1. Blank formulation

An experimental formulation of pharmaceutical suspension as per commercially available product was prepared for this study containing proprietary drug (0.05%), edetate disodium USP (0.01%), sodium chloride USP (0.86%), citric acid anhydrous USP (0.03%), and sodium citrate dihydrate USP (0.05%). Polysorbate 80 was omitted for validation purposes, making this formulation a polysorbate 80 vehicle (a pharmaceutical formulation containing all ingredients except polysorbate 80).

2.2.2.2. Sample preparation

Test solutions were prepared by addition of appropriate amounts of polysorbate 80 to the experimental formulation. These solutions were treated following the procedure described in Section 2.1.

2.2.2.3. Instrument and HPLC assay procedure

All samples were analyzed on a 1100 HPLC system with ChemStation (Hewlett-Packard, CA, USA). The separations were carried out on a 5 μm C_{18} column (Waters Symmetry C_{18}, 150×3.9 mm). The mobile phase was filtered before use. The chromatographic system employed a flow-rate of 1 ml/min, 15-μl injection volume, 20-min run time, and a spectrophotometric detector at 210 nm. After a stable baseline was established, replicate standards were injected to ensure reproducibility prior to sample analysis. System suitability criteria were established: The relative standard derivation of peak areas of six replicate injections, ≤5.0%; tailing factor, ≤2.0 and number of theoretical plates, >5000

based on oleic acid peak. A standard was inserted between six samples injections and at the end of the run.

3. Results and discussion

This method is based on the basic hydrolysis of polysorbate 80 in sample mixture followed by reversed-phase HPLC separation of oleic acid from other hydrolysis products and excipients (Fig. 2). The procedure is quick, simple and does not require time-consuming sample treatment. It could be used to directly analyze polysorbate 80 in pharmaceutical suspensions and could be applicable for the quantification of polysorbate 60 and polysorbate 40 in pharmaceutical solutions by determination of the quantity of stearic acid or palmitic acid, respectively.

In order to speed up the analysis, a quick and complete hydrolysis of polysorbate 80 was very desirable. The first hydrolysis was carried out using 1 *M* KOH in a 80 °C water bath. The hydrolysis was monitored by continuously taking samples from the hydrolysis container and injecting into a HPLC system. The analytical results showed that the hydrolysis could be completed in a couple of hours. Unfortunately, the analysis of method precision showed a RSD greater that 10% and the peak area of oleic acid, the targeted hydrolysis product, decreased with time, which hinted that milder conditions may be needed so that the hydrolysis product could be more stable.

When 0.1 *M* KOH was used and the hydrolysis temperature was kept at 80 °C, similar results were obtained. It was considered that the higher temperature resulted in the degradation of these hydrolysis products. Thus, the temperature was reduced to 40 °C. The reaction was monitored and the analytical results showed that the hydrolysis was completed in 6 h and the peak area of oleic acid did not show an obvious change within 8 h.

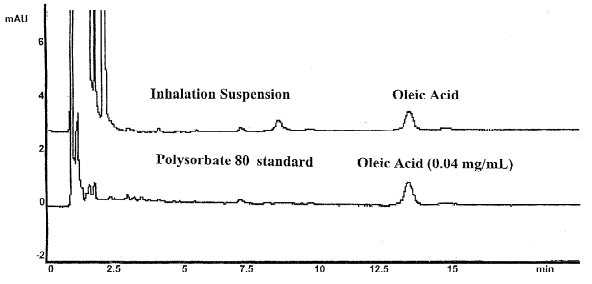


Fig. 2. Typical chromatograms of oleic acid for the polysorbate 80 standard and inhalation suspension.

Acidic hydrolysis of polysorbate 80 with HCl was tested as well. This hydrolytic reaction could be completed in 24 h, but inconsistent results were occasionally obtained.

Polysorbate 80 is a heterogeneous mixture of polysobitan with a range of oleic acids. The oleic acid yield due to hydrolysis could vary substantially from lot to lot. In this experiment, two lots of polysorbate 80 were examined. Samples of experimental formulation spiked with certain amounts of polysorbate 80 were hydrolyzed, and the hydrolyzed samples were analyzed against oleic acid standard. Calculations were based on the fact that 1 mol of polysorbate 80 could release 1 mol of oleic acid. The analytical results showed that hydrolysis of polysorbate 80 gave 90% and 98% yield, respectively. In order to reduce the bias on either high or low side during quantification, polysorbate 80 with a yield of oleic acid close to 100% was chosen as the standard.

Validation data were generated using the experimental formulation. Linearity was satisfactory. Seven-point vehicle standard curves (duplicate samples at seven different concentrations) were generated for the experimental formulation with concentration of polysorbate 80 ranging from 40 to 160% Label (0.02%, w/v polysorbate 80=100% Label). The curve obtained was linear (r^2=0.999) and the *y*-intercept was 1.667.

Method accuracy was carried out by analyzing three samples of blank formulation spiked with polysorbate 80 at 50 to 150% Label concentration of polysorbate 80 (0.02%, w/v polysorbate 80=100% Label). The average percentage recoveries of polysorbate 80 at the three spiked levels were 99.4, 100.9 and 101.1%, respectively.

Method precision data were generated for the experimental formulation samples and one commercial inhalation suspension and reproducible results were observed. Six samples gave a RSD of 0.6% for the experimental formulation and 1.0% for the commercial inhalation suspension.

4. Conclusion

This simple HPLC method presented above has been found selective, sensitive, precise, and accurate for analysis of polysorbate 80 in pharmaceutical suspensions. This procedure may also be suitable for quantification of other sorbitan esters by determination of the quantity of fatty acids released.

References

[1] A.H. Kibbe, Handbook of Pharmaceutical Excipients, Pharmaceutical Press, London, 2000, p. 511.
[2] A.S. Chawla, I. Hinberg, P. Blais, D. Johnson, Diabetes 34 (1985) 420.
[3] W.D. Loughheed, A.M. Albisser, H.M. Martindale, J.C. Chow, J.R. Clement, Diabetes 32 (1983) 424.
[4] Z.J. Twardowski, K.D. Nolph, T.J. McGary, H.L. Moore, Am. J. Hosp. Pharm. 40 (1983) 579.
[5] E.G. Browna, T.J. Hayes, Analyst 80 (1955) 755.
[6] N.T. Crabb, H.E. Persinger, J. Am. Oil Chem. Soc. 41 (1964) 752.
[7] R.A. Greff, E.A. Setzkorm, W.D. Leslie, J. Am. Oil Chem. Soc. 42 (1965) 180.
[8] A. Nozawa, T. Ohnuma, S. Tatsuya, Analyst 101 (1976) 543.
[9] N.H. Anderson, J. Girling, Analyst 107 (1982) 836.
[10] T.H. Tani, J.M. Moore, T.W. Patapoff, J. Chromatogr. A 786 (1997) 99.
[11] M. Puttmann, H. Krug, E. von Ochsenstein, R. Katterman, Clin. Chem. 39 (1993) 825.
[12] R. Abushufa, P. Reed, C. Weinkove, Clin. Chem. 40 (1994) 1707.
[13] M.I. Aveldano, M. VanRollins, L.A. Horrocks, J. Lipid Res. 24 (1983) 83.
[14] A. Mehta, A.M. Oeser, M.G. Carlson, J. Chromatogr. B Biomed. Sci. Appl. 719 (1998) 9.
[15] Y. Hori, K. Nakamura, M. Yamamoto, K. Shimada, H. Nakadaira, N. Shibuya, K. Endoh, K. Ogoshi, Ann. Clin. Biochem. 35 (1998) 279.
[16] M.I. Aveldano, L.A. Horrocks, J. Lipid Res. 24 (1983) 1101.
[17] M. Ikeda, K. Shimada, T. Sakaguchi, J. Chromatogr. 272 (1983) 251.
[18] M. Weiss, M. Manneberg, J.F. Juranville, H.W. Lahm, M. Fountoulakis, J. Chromatogr. A 795 (1998) 263.
[19] K.L. Woo, J.I. Kim, J. Chromatogr. A 862 (1999) 199.
[20] Z. Oszi, G. Petho, J. Pharm. Biomed. Anal. 18 (1998) 715.