American Journal of Pharmacology & Therapeutics

Research Article

Docetaxel Formulation in Polysorbate 80: A Complex and Sensitive System

Jean-René Authelin[1]*, Doris Andert[2], Lucile Boilait[3], Jean-Jacques Nguyen-Huu[1], Yan-Yan Zhang[4] and Ping Ni[1]

[1]*Sanofi R&D, 94400 Vitry sur Seine, France*

[2]*Sanofi Industrial Affairs, 65926 Frankfurt am Main, Germany*

[3]*Sanofi R&D, 34000 Montpellier, France*

[4]*Sanofi R&D, 200040 Shanghai, China*

***Address for Correspondence:** Jean-René Authelin, Sanofi R&D, 94400 Vitry sur Seine, France, E-mail: jean-rene.authelin@sanofi.com

Submitted: 21 September 2020; **Approved:** 25 September 2020; **Published:** 30 September 2020

Cite this article: Authelin JR, Andert D, Boilait L, Nguyen-Huu JJ, Zhang YY, Ni P. Docetaxel Formulation in Polysorbate 80: A Complex and Sensitive System. Am J Pharmacol Ther. 2020 Sep 30;4(1): 001-006. doi: 10.37871/ajpt.id19

ABSTRACT

Background: Innovator docetaxel (in Taxotere reference product), a poorly water-soluble agent with a narrow therapeutic index, is developed as a solution in Polysorbate 80 and ethanol. Such formulations form metastable micelles and are considered complex parenterals. Formulation process control impacts quality attributes efficacy and safety. Characterization of micellar systems in infusion bags is required to ensure good biopharmaceutical performance and equivalence of docetaxel generic formulation.

Methods: HPLC, GC, water content (Karl Fischer) and pH were used to characterize chemical properties and oxygen content in the vial headspace. Dynamic light scattering was used for micelle size. Free drug was measured with a 14 kD dialysis bag membrane and quantified in external phase with UPLC.

Results: Significant differences in impurity profiles (*vs* reference product) were found. Compared with originator, ethanol content was much higher in both generics; pH was significantly higher for one generic. The headspace of one generic revealed residual oxygen, indicating either absence of blanketing by nitrogen or poor tightness of vial closures. Micelle size of the generics was similar to originator; during dilution in saline solution, docetaxel crystals appeared in all samples but much more massively in one generic, depicting much lower physical stability. In dialysis tests both generics exhibited free drug level ~20% less than the originator.

Conclusion: Both generic docetaxel exhibited significant differences relative to the innovator docetaxel. Further studies are warranted to determine clinical equivalence of these generic formulations. Micellar formulations merit specific attention in regulatory bioequivalence guidance to increase availability of quality products to patients.

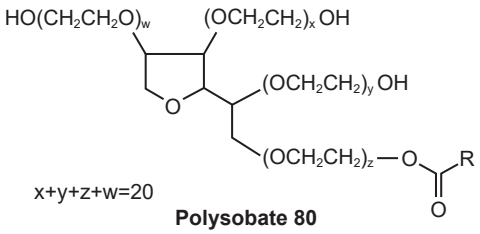
Keywords: Docetaxel; Generic drugs; Formulation; Bioequivalence; Polysorbate 80 micelles

INTRODUCTION

Docetaxel, a widely used antineoplastic agent in the taxoid family, is indicated for treatment of breast, non-small cell lung, prostate, head and neck, and gastric cancers as a single agent and in combination therapies [1,2]. The highly lipophilic nature of docetaxel requires formulation of the drug product as a solution in the surfactant polysorbate 80. Polysorbate 80 (PS80) is a nonionic surfactant [3]. The innovator docetaxel (Taxotere formulation) is only formulated with polysorbate 80 and ethanol [1].

Generic formulations of docetaxel have been available for more than a decade in several countries. Registered generics either copy the innovator docetaxel formulation or may use other solubilizing agents, especially polyethylene glycol. All formulations include polysorbate 80 which is a sugar extensively modified by polyoxyethylene substitution and esterification in figure 1 [4]. Such formulations form micelles when mixed in an aqueous solution, eg., an infusion bag for intravenous use [5]. The micelle system is metastable, and after some latency period, docetaxel crystals will inevitably precipitate [6]. Storage and handling instructions in the label for the two-vial formulation of docetaxel are designed to minimize product degradation and the release of drug from the micelles [1,6]. Taxotere one-vial formulation was developed to further reduce the risk of crystallization [6].

Micelles persist for some time in blood and carry most of the drug, while the remainder exists in an equilibrium between free drug and docetaxel bound to plasma proteins [7]. It is likely that relevant polysorbate 80 micelles *in vivo* are rapidly cleared [8]. Polysorbate 80 exists in different grades, with different chemical and physical properties [9-11]. The binding of docetaxel to plasma proteins displays a non-linear behavior when the amount of polysorbate 80 is varied while docetaxel concentration is unchanged [12]. It has been suggested that generics having different levels of polysorbate 80



Polysobate 80

Fatty acid ester	Content in PS80 (%)	Chemical formula of R
Myristic (C_{14})	≤5	$(CH_2)_{12}CH_3$
Palmitic (C_{16})	≤16	$(CH_2)_{14}CH_3$
Palmitoleic ($C_{16:1}$)	≤8	$(CH_2)_7CH=CH(CH_2)_5CH_3$
Stearic(C_{18})	≤6	$(CH_2)_{16}CH_3$
Oleic ($C_{18:1}$)	≥58	$(CH_2)_7CH=CH(CH_2)_7CH_3$
Linoleic ($C_{18:2}$)	≤18	$(CH_2)_7CH=CH(CH2)CH=CH(CH_2)_4CH_3$
Linolenic ($C_{18:3}$)	≤4	$(CH_2)_7CH=CH(CH_2)CH=CH(CH_2)CH=CH(CH_2)CH_3$

Figure 1: Generic chemical structure of polysorbate 80 and corresponding fatty acid content. Polysorbate 80 (PS80) is a mixture of partial esters of fatty acids, mainly oleic acid, with sorbitol and its anhydrides ethoxylated with approximatively 20 moles of ethylene oxide for each mole of sorbitol and sorbitol anhydrides [4].

DOI: https://dx.doi.org/10.37871/ajpt.id19

with different percentages of fatty acid esters or other additives could potentially result in variations in pharmacodynamics or adverse event profiles [12]. A retrospective study of five docetaxel products showed significant differences in the adverse event profiles that were related to the level and type of additives present, with polysorbate 80 amounts in particular having a negative association with non-hematologic adverse events, suggesting low levels of polysorbate 80 may result in reduced solubilization of the drug and increased adverse events [3]. Another study found significantly increased hematologic and cutaneous toxicities and treatment discontinuations in patients treated with generic versus original docetaxel [13]. Given that micelles play a role in delivery and pharmacokinetics, any parameter that affects the physical chemistry of micelles could ultimately have an impact on the efficacy and safety of micelle-formulated drugs.

According to the European Medicines Agency guidance on the investigation of bioequivalence, intravenous micellar injections together with liposomes and emulsion dosage forms can be regarded as complex parenterals, and thus a bioequivalence study cannot be systematically waived [14]. Further regulatory discussions in Europe about such products have led the European Medicine Agency to recommend performing an in-depth characterization of the micellar system in infusion bags in order to ensure good biopharmaceutical performance [15]. A bioequivalence study may be required unless both products contain the same excipients in very similar quantity and it can be adequately justified that any quantitative difference in the comparative results does not affect surrogate markers of bioequivalence; its relevance to the clinical setting should also be discussed [15].

Other regulatory bodies are also evaluating requirements for the approval of parenteral generic drugs. As one of the largest generic drug markets in the world, China has proposed new, retroactive evaluation and approval requirements for both domestic and imported generic drugs. Given the current industry-wide focus of the Chinese Health Authorities on Generic Quality and Consistency Evaluation (GQCE), our study assessed two lead docetaxel Chinese generics, in particular their micellar systems, in comparison with the innovator docetaxel drug product [16].

MATERIALS AND METHODS

Chinese docetaxel generics marketed by Jiangsu Hengrui Medicine Co., Ltd. ("Hengrui") and Qilu Pharmaceutical Co., Ltd. ("Qilu") were compared with the innovator docetaxel drug product by High Performance Liquid Chromatography (HPLC) retention times to confirm product identity and to compare levels of impurities. Gradient reversed-phase HPLC was used to determine docetaxel content versus standard solutions. Ethanol content was determined by Gas Chromatography (GC), water content by Karl Fischer titration, and pH measurements were used to characterize product chemical properties. Oxygen content in the headspace of the vial and fill volume were also determined. The solvents provided for product reconstitution were compared visually, for fill volume, and ethanol content by GC.

Dynamic light scattering was used to characterize micelle size after the drug product was diluted to different concentrations (from 0.5 mg/mL to 0.005 mg/mL) in a saline solution. Free drug, i.e., not bound to micelles, was determined by allowing a 0.5 mg/mL drug product infusion in saline solution to equilibrate with pure saline solution passed through the 14 kD membrane of a dialysis bag. Free docetaxel was quantified in the external phase using gradient reversed-phase Ultra Performance Liquid Chromatography (UPLC).

RESULTS

Docetaxel content in both Hengrui and Qilu was 96% of that expected. The chemical analysis of generics from these two Chinese manufacturers showed significant differences in the impurity profile in comparison with the innovator drug product, including unknown impurities, particularly in Qilu in figure 2. The impurity at relative retention time 1.29 for Qilu is out of specification (OOS; based on USP specification for Docetaxel Injection) [17].

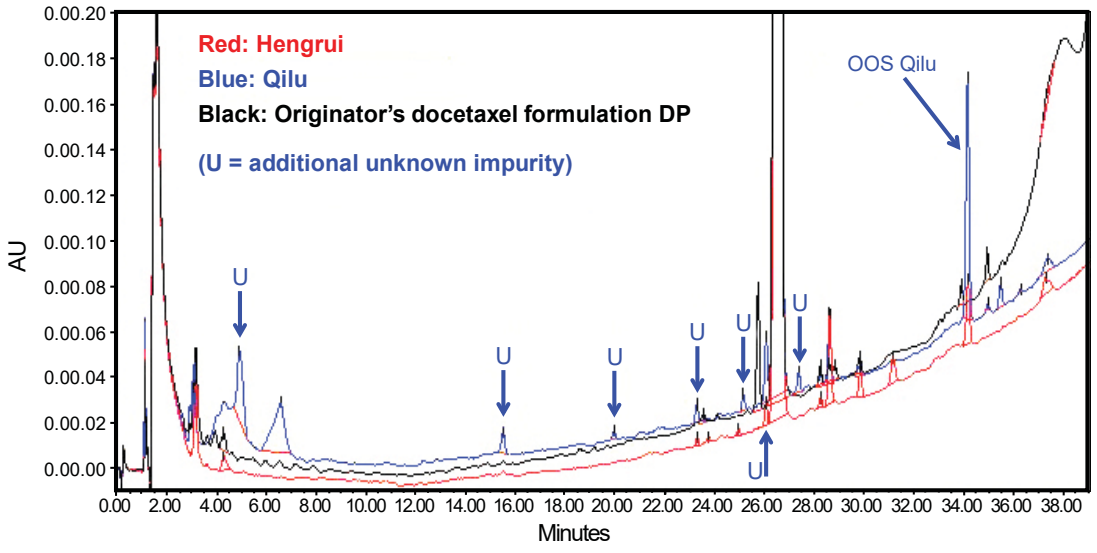


Figure 2: Impurity profile of Hengrui and Qilu compared with innovator docetaxel drug product.
AU: Absorbance Units; DP: Drug Product; OOS: Out of Specification; U: additional unknown impurity

DOI: https://dx.doi.org/10.37871/ajpt.id19

The pH was significantly higher for Hengrui (4.8 versus 3.6 for the innovator), which is known to increase the chemical degradation; the pH of Qilu was 3.2. The headspace of Hengrui revealed residual oxygen in the head space of 5 vials tested (5.6% to 18.7% volume% O_2 versus 0.2% to 0.8% for Qilu), indicating either the absence of blanketing by nitrogen or poor tightness of vial closures for Hengrui. Water content in both generics were the same as that of the innovator drug product (0.1%). Ethanol content, on the other hand, was much higher in both generics (1.3% for Hengrui, 0.9% for Qilu) compared to the innovator (< 0.1%).

The micelle size of the generics was similar (10.6 nm) to that of the innovator docetaxel drug product. During dilution in saline solution, docetaxel crystals appeared in all samples, as the system is metastable. However, a population of particles ranging from micron size to tenths of micron size, appeared massively in Hengrui, indicating a much lower physical stability in figure 3. In the dialysis tests, after a 4 hour infusion both generics exhibited a level of free drug passing through the dialysis membrane that was approximately 20% less than the innovator in figure 4.

DISCUSSION

For oncologic agents like docetaxel, with a narrow therapeutic window, accurate dosing is essential for efficacy and safety. In a previously reported study assessing the pharmaceutical quality of docetaxel generics versus the originator drug product, Hengrui and Qilu were among the 31 commercially-available generic formulations of docetaxel available in 14 countries in Asia, Africa, the Middle East, and Latin America, that were analyzed for docetaxel content, impurity levels, and pH versus the innovator docetaxel [18]. This early study found 90% of the generics did not meet specified quality criteria, having either a lower than expected amount of docetaxel and/or a high level of impurities. Moreover, both Hengrui and Qilu contained < 90% of the expected docetaxel content. Though total impurities in Hengrui exceeded 3%, the impurities were not identified, and thus it is unknown whether they resulted during manufacture or subsequent degradation, eg., association with the observed variations in pH. In addition, this early study did not examine the micellar formulations of these docetaxel generics [18].

In the present study, the docetaxel content for the generics was within expected levels. Hengrui had a higher pH and lower physical stability compared with the innovator drug product, material from both Hengrui and Qilu had higher levels of impurities, and Qilu was out of USP specification for Docetaxel Injection for single unknown impurity levels. Nonclinical impurity studies to evaluate the potential impact of the difference in impurity profiles would need to be provided. According to International Conference on Harmonisation (ICH) guidance, these studies should include qualification, i.e., establishing safety of degradation products or degradation profile, justification of degradation product levels, and structural identification of impurities present in the new drug substance and in the new drug product higher than defined thresholds [19,20]. Residual oxygen in the head space revealed either absence of nitrogen blanketing or poor vial closure tightness for Hengrui, which is of importance because both docetaxel and polysorbate are known to be sensitive to oxidation [10,11]. All of these differences between innovator drug product as well as the potential for degradation and the lower levels of free drug passing

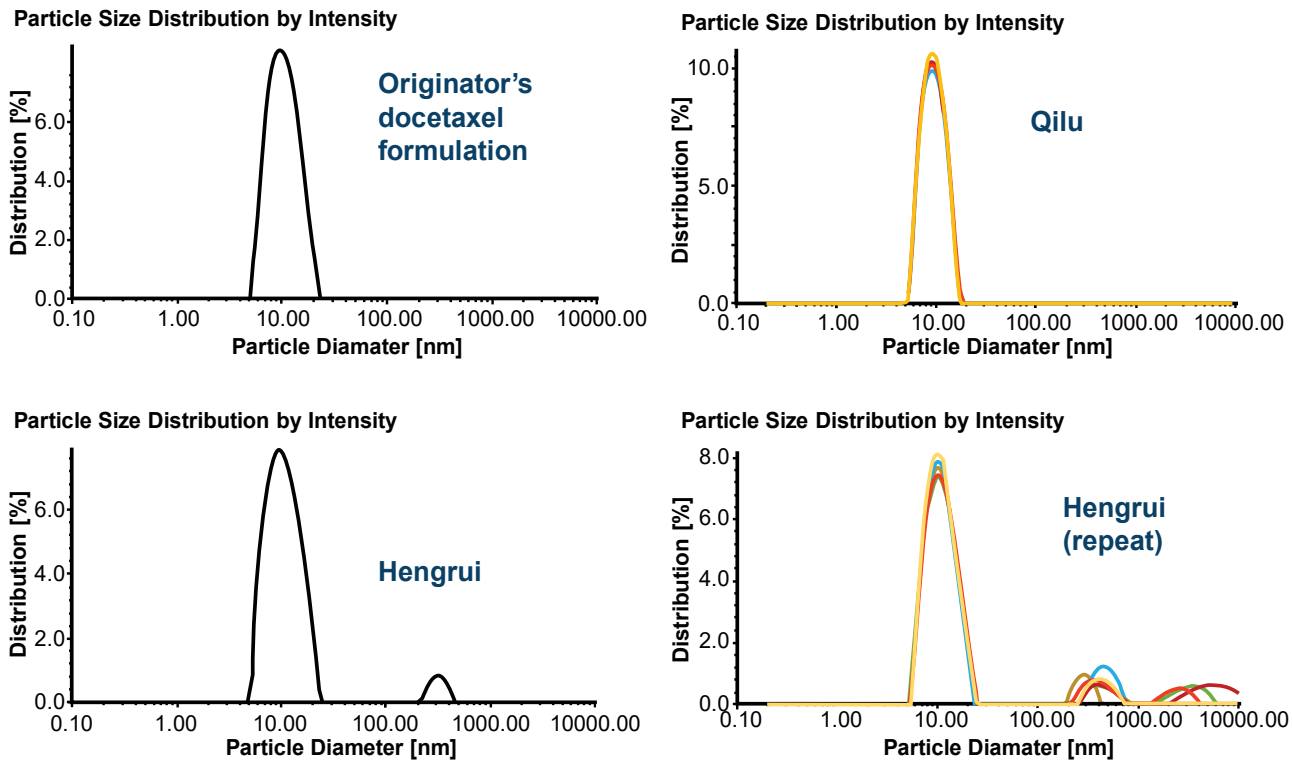


Figure 3: Dynamic light scattering reveals precipitation of docetaxel for first dilution level for Hengrui (0.5 mg/mL), indicating a lower physical stability.

DOI: https://dx.doi.org/10.37871/ajpt.id19

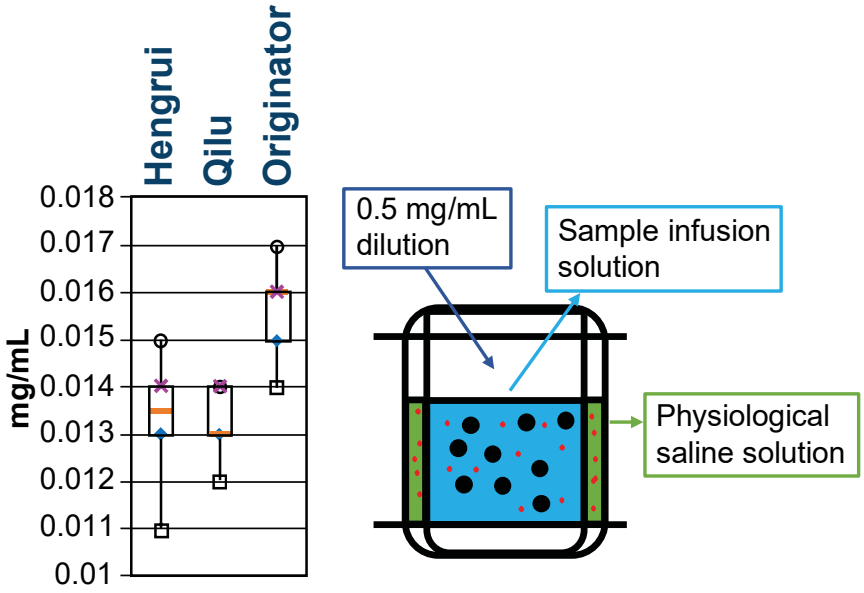


Figure 4: Box plot of free drug for innovator docetaxel formulation, and for Hengrui and Qilu diluted after 4 hours infusion. The amount of drug passing the dialysis membrane is significantly less for generics compared with the innovator drug product.

through the dialysis membrane seen with the generics, particularly Hengrui, may have an impact on the Pharmacokinetic (PK) profiles of the generic drugs *in vivo*. In addition, the PK of unbound docetaxel appears to have a greater impact on overall Pharmacodynamic (PD) / adverse effects than total drug levels [21]. Its level is regulated by the complex dynamic equilibrium of docetaxel between micelles, plasma proteins (including albumin, lipoproteins, and α1-Acid Glycoprotein [AAG]), cells and the metabolization/ clearance rate of the drug. Although micelles may break surfactant to high dilution at the beginning of the infusion, they may persist or reform later as the polysorbate blood concentration will reach levels well above the critical micellar concentration [15]. The lower level of free docetaxel observed with generics, particularly Hengrui, in the *in vitro* dialysis test, indicates that the micelle-to-aqueous phase drug substance partition is lower for generics. The free docetaxel level of the generics may change during dialysis; this could be expected to occur as well in the bloodstream, where the concentration of free docetaxel is also affected by the concentration of PS80 and binding to plasma proteins [12]. Considering that formulated docetaxel has a narrow therapeutic index, the complex PK/PD interactions between docetaxel and the excipient may affect safety and possibly efficacy [22].

According to the European Guideline on the Investigation of Bioequivalence, there are three biowaiver criteria: (1) rapid disassembly of the micelle on dilution occurs and the drug product is not designed to control release or disposition; (2) the method and rate of administration is the same as the currently approved product; (3) the excipients do not affect the disposition of the drug substance [14]. The guidelines further stipulate: "In these cases, the composition of the micelle infusion, immediately before administration, should be qualitatively and quantitatively the same as that currently approved and satisfactory data should be provided to demonstrate similar physicochemical characteristics, for example, the critical micelle concentration, the solubilisation capacity of the formulation (such as Maximum Additive Concentration), free and bound active substance and micelle size [14]." Subsequently, the European Medicine Agency clarified in a specific concept paper thve importance of characterizing the micellar system in infusion bags to ensure good biopharmaceutical performance, which may necessitate bioequivalence studies [15]. The differences between docetaxel generics and the innovator docetaxel found in the present study relate directly to whether it is reasonable to systematically waive requirements for bioavailability studies of such substances solubilized in micellar systems.

CONCLUSION

In summary, our study found that the generics made by Hengrui and Qilu exhibited significant differences in physical chemistry profile, physical stability, and free drug from the original drug product docetaxel. Further studies to determine the clinical implications of these findings are necessary. Moreover, data equivalence requirements, such as including micellar characterization to support a biowaiver for generic complex formulations of drugs having a narrow therapeutic index like docetaxel, may be beneficial. Given the ongoing GQCE discussion in China, these types of specific equivalence requirements may also merit specific attention in regulatory guidance to increase the availability of quality products to patients.

ACKNOWLEDGMENTS

Funding was provided by Sanofi. The authors would like to acknowledge Dorothee Semiond for her contribution to this research. The authors thank Phillips Gilmore Oncology Communications for editorial assistance in preparation of the manuscript.

REFERENCES

1. Taxotere (docetaxel) injection concentrate, intravenous infusion (IV). Prescribing information. Sanofi-aventis US LLC, Bridgewater, NJ. 2010 May. https://tinyurl.com/yxjasahk

DOI: https://dx.doi.org/10.37871/ajpt.id19

2. Taxotere. Summary of product characteristics. Aventis Pharma SA, Antony, France. https://tinyurl.com/yxqc4w2s

3. Tagawa N, Sugiyama E, Tajima M, Sasaki Y, Nakamura S, Okuyama H, Shimizu H, Sato VH, Sasaki T, Sato H. Comparison of adverse events following injection of original or generic docetaxel for the treatment of breast cancer. Cancer Chemother Pharmacol. 2017 Oct;80(4):841-849. doi: 10.1007/s00280-017-3425-3. Epub 2017 Sep 1. PMID: 28864856.

4. European Pharmacopoeia. 10th Edition. European Directorate for the Quality of Medicines and Healthcare. Council of Europe, Strasbourg, France. July 2019. https://tinyurl.com/y5534wz5

5. Schwartzberg LS, Navari RM. Safety of Polysorbate 80 in the Oncology Setting. Adv Ther. 2018 Jun;35(6):754-767. doi: 10.1007/s12325-018-0707-z. Epub 2018 May 23. PMID: 29796927; PMCID: PMC6015121.

6. Hart M, Acott S. Physical and chemical stability of Taxotere (docetaxel) one-vial (20 mg/ml) infusion solution following refrigerated storage. Ecancermedicalscience. 2010;4:202. doi: 10.3332/ecancer.2010.202. Epub 2010 Dec 15. PMID: 22276044; PMCID: PMC3234020.

7. Loos WJ, Baker SD, Verweij J, Boonstra JG, Sparreboom A. Clinical pharmacokinetics of unbound docetaxel: role of polysorbate 80 and serum proteins. Clin Pharmacol Ther. 2003 Oct;74(4):364-71. doi: 10.1016/S0009-9236(03)00222-4. PMID: 14534523.

8. Webster LK, Linsenmeyer ME, Rischin D, Urch ME, Woodcock DM, Millward MJ. Plasma concentrations of polysorbate 80 measured in patients following administration of docetaxel or etoposide. Cancer Chemother Pharmacol. 1997;39(6):557-60. doi: 10.1007/s002800050615. PMID: 9118471.

9. Braun AC, Ilko D, Merget B, Gieseler H, Germershaus O, Holzgrabe U, Meinel L. Predicting critical micelle concentration and micelle molecular weight of polysorbate 80 using compendial methods. Eur J Pharm Biopharm. 2015 Aug;94:559-68. doi: 10.1016/j.ejpb.2014.12.015. Epub 2014 Dec 13. PMID: 25513959.

10. Kerwin BA. Polysorbates 20 and 80 used in the formulation of protein biotherapeutics: structure and degradation pathways. J Pharm Sci. 2008 Aug;97(8):2924-35. doi: 10.1002/jps.21190. PMID: 17973307.

11. Kranz W, Wuchner K, Corradini E, Berger M, Hawe A. Factors Influencing Polysorbate's Sensitivity Against Enzymatic Hydrolysis and Oxidative Degradation. J Pharm Sci. 2019 Jun;108(6):2022-2032. doi: 10.1016/j.xphs.2019.01.006. Epub 2019 Jan 11. PMID: 30639738.

12. Marquez M, Quintanar L, Hernandez G. Characterization of plasma protein binding and the effect of the vehicle of docetaxel formulations. J Mex Chem Soc. 2017;61(4):370-379. https://bit.ly/2EGnKaK

13. Elm'hadi C, Tanz R, Khmamouche MR, Toreis M, Mahfoud T, Slimani KA, Errihani H, Ichou M. Toxicities of docetaxel: original drug versus generics-a comparative study about 81 cases. Springerplus. 2016 Jun 16;5(1):732. doi: 10.1186/s40064-016-2351-x. PMID: 27386229; PMCID: PMC4909666.

14. European Medicines Agency. Guideline on the investigation of bioequivalence. January 2010. Accessed March 2, 2020. https://bit.ly/33eCBTw

15. European Medicines Agency. Reflection paper on the pharmaceutical development of intravenous medicinal products containing active substances solubilized in micellar systems. 2012 March. https://bit.ly/3mYQOeZ

16. Lexology. Generic consistency evaluation in China: the coming paradigm shift in drug pricing and a patent cliff. 2020 March. https://www.lexology.com/library/detail.aspx?g=8721c9d6-124c-4053-864d-2f90e299bed7

17. United States Pharmacopeial Convention. Docetaxel Injection. May 2012. https://bit.ly/2Gcqqxw

18. Vial J, Cohen M, Sassiat P, Thiébaut D. Pharmaceutical quality of docetaxel generics versus originator drug product: a comparative analysis. Curr Med Res Opin. 2008 Jul;24(7):2019-33. doi: 10.1185/03007990802207874. Epub 2008 Jun 9. PMID: 18544188.

19. ICH Topic Q 3 A (R2). Impurities in new drug substances. Note for guidance on impurities testing: impurities in new drug substances. October, 2006. https://bit.ly/2EIGmHj

20. ICH Topic Q 3 B (R2). Impurities in new drug substances. Note for guidance on impurities in new drug products. June 2006. https://bit.ly/3cFTdX4

21. Minami H, Kawada K, Sasaki Y, Igarashi T, Saeki T, Tahara M, Itoh K, Fujii H. Pharmacokinetics and pharmacodynamics of protein-unbound docetaxel in cancer patients. Cancer Sci. 2006 Mar;97(3):235-41. doi: 10.1111/j.1349-7006.2006.00166.x. PMID: 16542221.

22. Bissery MC, Nohynek G, Sanderink GJ, Lavelle F. Docetaxel (Taxotere): a review of preclinical and clinical experience. Part I: Preclinical experience. Anticancer Drugs. 1995 Jun;6(3):339-55, 363-8. doi: 10.1097/00001813-199506000-00001. PMID: 7670132.

DOI: https://dx.doi.org/10.37871/ajpt.id19