Comparison of Chemical Behavior of Original and Generic Docetaxel Formulations as Non-alcoholic Preparations: Discussion about Diluent Solvents for Docetaxel

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Although generic anti-tumor agents are in wide clinical use, they have not in all cases been shown to be equivalent to the original agents after preparation. In the present study, original and generic docetaxel formulations were compared with respect to stability when prepared as a non-alcoholic solution for use. When the original formulation was diluted with physiological saline solution to make a non-alcoholic preparation, the concentration decreased with time, whereas no such decrease occurred when a preparation of the generic formulation was made in a similar manner. With both the original and generic formulations, no decrease in docetaxel concentration with time was found after dilution with 5% glucose solution. On the basis of these results, it is concluded that the behaviors of original and generic docetaxel formulations are not equivalent when prepared, but that the original and generic formulations can be taken to be equivalent if they are diluted with 5% glucose solution at preparation.

Key words—docetaxel; stability; diluent solvent; generic; non-alcoholic docetaxel; micelle

INTRODUCTION

Docetaxel hydrate (DTX), with the commercial name “Taxotere®”, is a semisynthetic, taxoid anti-tumor agent that was created in 1984, with 10-deacetylbaccatin III [Fig. 1 (A)], a substance derived extracted from leaves of the European yew, as the precursor substance. DTX promotes tubulin polymerization, stabilize microtubules, and also inhibits tubulin depolymerization, eliciting anti-tumor effects [Fig. 1 (B)]. In Japan, it was approved in 1996, and as of 2017 it is in wide use for treating various solid cancers, including breast cancer, and also non-small-cell lung cancer, gastric cancer, head and neck cancer, ovarian cancer, esophageal cancer, uterine corpus cancer, and prostate cancer. Although immune-checkpoint-inhibitors based on novel mechanisms of action have been developed since 2015, DTX has been used in the standard treatment groups in both Japanese and overseas clinical studies of anti-cancer agents.1-4) The DTX preparations used are for intravenous infusion, and are of two types, one, original docetaxel (OR-DTX), is prepared by dissolving the formulation in the solvent (13% ethanol) included with the DTX, and the other requiring no premixing. OR-DTX is prepared by dissolving in the included solvent, and administration to alcohol-hypersensitive patients is therefore problematic. With the latter formulation, on the other hand, physiological saline solution (PSS) or 5% glucose solution in distilled water (5%Glu) is used instead of the included solvent. This enables administration to alcohol-hypersensitive patients, whereas its disadvantage is that preparation requires considerable skill and time.

Non-alcoholic formulations of generic DTX (GE-non-alc-DTX) have now been launched by a number of pharmaceutical companies, and since 2015 a DTX intravenous infusion, Yakult Honsha Co., Ltd., has been in use. Since the formulation of Yakult is widely used clinically, we used the formulation as typical among all generic formulations. The characteristics of OR-DTX and GE-non-alc-DTX are shown in Table 1. GE-non-alc-DTX has various advantages in comparison with OR-DTX, including that it is less expensive, it is a shrink-wrapped formulation that was developed with attention given to prevention of workplace exposure, and it is a non-alcoholic formulation.

Although basic data must be verified in order to establish the safety of generic drugs, no research has been conducted previously comparing the chemical
and biological behaviors of OR-DTX and GE-non-alc-DTX. OR-DTX is usually diluted with the attached medium and prepared as an alcoholic solution. However, in order to administer OR-DTX to alcohol-hypersensitive patients, OR-DTX is diluted with either PSS or 5% Glu to prepare a non-alcoholic preparation. The stability of OR-DTX in a non-alcoholic solution has not been compared to that of GE-non-alc-DTX. In the present study, we established the analytical method of DTX using HPLC with higher separation performance than under the analysis conditions specified in the OR-DTX Interview Form (version 11), and the stability of DTX in OR-DTX and GE-non-alc-DTX was evaluated after dissolving in PSS or 5% Glu.

**METHODS**

**Study Formulations** Commercial products of Taxotere® intravenous infusion 20 mg (lot no. 6F034A; Sanofi SA, Tokyo) and Yakult docetaxel intravenous infusion (lot no. DKAAJA; Yakult Honsha Co., Ltd., Tokyo) were purchased for OR-DTX, and, for GE-non-alc-DTX, respectively. At Tokyo Medical Center, there are numerous clinical departments where alcohol is not used as the solvent and the product is instead dissolved directly in the diluent sol-
vent so that drugs can be administered even to alcohol-hypersensitive patients. Therefore, in accordance with clinical practice, non-alcoholic preparations of OR-DTX (OR-non-alc-DTX) were made by dissolving directly in PSS or 5% Glu to the specified concentration of 2 mg/mL.

Test Samples and Reagents  Butyl parahydroxybenzoate (lot no. KPM0471; Wako Pure Chemical Industries, Ltd., Osaka) was used as the internal standard (IS). Acetonitrile, methanol, 1,2-dichloroethane, and sodium acetate (lot no. CKE1536) of special-reagent grade or for HPLC were purchased from Wako Pure Chemical Industries. For preparation of 0.1 mol/L acetic acid buffer, at pH 4.0, glacial acetic acid (Nikko Chemicals Co., Ltd., Gifu), sodium acetate (Wako Pure Chemical Industries), and distilled water for injection (Hikari Co., Ltd., Tokyo) were used. As the standard substances, DTX trihydrate (STD-DTX trihydrate; lot no. 15070B001; AdooQ Bioscience, Irvine), DTX anhydrate (STD-DTX anhydrate; lot no. X54WA-BS; Tokyo Chemical Industry Co., Ltd., Tokyo), 10-deacetylbaccatin III (lot no. 4U7XH-QG; Tokyo Chemical Industry Co., Ltd.), Polysorbate 80 (lot no. 706B4070; Kanto Chemical Co., Inc., Tokyo), and polyethylene glycol 400 (lot no. 704B2143; Wako Pure Chemical Industries), were used.

Preparation of Test Samples  In separation experiments with each formulation of OR-DTX and GE-non-alc-DTX, 100 μL of each solution was dissolved in PSS or 5% Glu and the formulation was taken, and 800 μL of PSS or 5% Glu was added. In addition, STD-DTX trihydrate and STD-DTX anhydrate were dissolved in ethanol, and 100 μL of IS was added to 900 μL of the resulting solution. For the IS, 5 mg of butyl parahydroxybenzoate was dissolved in 5 mL of ethanol.

Particle diameters were measured by the dynamic light scattering method. Each formulation was diluted in PSS or 5% Glu, and the solution was adjusted to a concentration of 2.0 mg/mL.

HPLC Conditions  The HPLC equipment consisted of a pump (LC-10AS; Shimadzu Corporation, Tokyo), a detector (SPD-M10A VP Shimadzu Corporation), and a column (TSKgel® ODS-100Z; particle diameter: 3 μm; tube diameter: 4.6 mm; tube length: 250 mm; Tosoh Corporation, Tokyo). All data were stored on a personal computer, using a network repeater (CBM-10A; Shimadzu Corporation), and waveform analysis was carried out using Class-LC10 (Shimadzu Corporation). For injection, 20 μL samples were injected using a 7125 valve (Rheodyne LLC, Rohnert Park), and measurement was conducted, after 0, 4, 12, 24, 48, and 72 h.

We modified the mobile phase composition which is referred to in the interview form 12th edition. The solutions used for the HPLC mobile phase were solution 1, which was a 20 : 60 : 20 (v/v/v) mixture of 0.1 mol/L acetic acid buffer (pH 4.0), methanol, and acetonitrile; and solution 2, which was a 10 : 60 : 20 (v/v/v/v) mixture of 0.1 mol/L acetic acid buffer (pH 4.0), methanol, acetonitrile, and 1,2-dichloroethane. Solution 1 was replaced with solution 2 by the stepwise elution method, over a 16-min period after elution of the DTX peak. The mobile phase flow rate was 1.5 mL/min, and the column temperature was 30°C. Detection of 10-deacetylbaccatin III, IS, DTX, and Polysorbate 80 was conducted using a multi-wavelength detector, mainly at an ultraviolet wavelength of 230 nm. Under these conditions, the retention times of DTX and IS were approximately 13 and 8 min, respectively.

Calibration Curve  Quantification was conducted by the IS method. The DTX standard substance was dissolved in ethanol, and a 2.5 mg/mL solution was prepared. This was then used to prepare solutions diluted 2-, 10-, 20-, and 100-fold. A 1.0 mg/mL alcoholic solution was used for the IS. The calibration curve showed a high degree of linearity, at \( R^2 = 0.99558 \).

Peak Purity Test  For the HPLC peak purity test, the spectrum contrast method was used, with the Class-LC10 algorithm. In this method, two spectra, \( S_1 \) and \( S_2 \), are compared, and taking the corresponding vectors to be \( \vec{S}_1 \) and \( \vec{S}_2 \), if the two spectra have similar waveforms, the two vectors have the same orientation, and the two spectra are thus judged to be the same.

Particle Diameter Measurement by the Dynamic Light Scattering Method  Each formulation was dissolved in PSS or 5% Glu, and then filtered with a pore size of 0.45 μm. The micelle particle diameters were measured by the dynamic light scattering method, using a zeta potential, particle size, and molecular weight analysis system (ELSZ-2000; Otsuka Electronics Co., Ltd., Osaka).

Cloud Point Measurement  Polysorbate 80 (lot no. D5JEC; Tokyo Chemical Industry Co., Ltd., Tokyo) and polyethylene glycol 400 (lot no.
**RESULTS**

**DTX Separation**  Figure 2(A) shows chromatograms of (1) IS, (2) DTX, and (3) Polysorbate 80, Fig. 2(B) shows the DTX absorption spectrum, and Fig. 2(C) shows the DTX peak purity curve. A typical HPLC chromatogram of OR-non-alc-DTX dissolved in 5% Glu is shown in Fig. 2(A). Since the peak purity values obtained in the DTX peak purity test were approximately 1 [Fig. 2(C) and Table 2], separation is considered to have been almost complete. The chromatograms of GE-non-alc-DTX and STD-DTX were almost the same as that of OR-DTX.

Table 2 shows the retention times, column affinities, peak purities, and tailing factors of 10-deacetylbaccatin III, IS, DTX, and Polysorbate 80. Since DTX was produced from the semisynthetic precursor 704B2143; Wako Pure Chemical Industries) were each prepared in PSS or 5% Glu to make 5.4% (w/v) solutions that is equivalent to the concentration of Polysorbate 80 contained in GE-DTX. Citric acid was added to these solutions to make concentrations of 0%, 0.1%, 0.5%, 1%, 2%, and 5% (w/v), and 10 mL of each of these was warmed, while stirring with a magnetic stirrer, and the temperature at onset of turbidity was determined.

**Statistical Analysis**  Statistical analysis of the proportion of drug remaining was carried out by one-way analysis of variance (Tukey’s HSD test), using the statistical analysis software IBM SPSS Statistics (version 24; IBM Japan, Ltd.; Tokyo).

Using the mobile phase solvent with increased separation performance that the present authors have established, contour graphs suggested that each substance can be separated without superposition. Since OR-non-alc-DTX, STD-DTX trihydrate, and STD-DTX anhydrate all had peaks with the same retention times, the peak at 12.8 min is considered to be DTX. This notion was further supported by the fact that the peak had a maximum absorption wavelength of approximately 230 nm which was the same as those for STD-DTX trihydrate and STD-DTX anhydrate because they dissolve in the same form as DTX molecule, and its purity was approximately 1.

Table 2 shows the retention times, column affinities, peak purities, and tailing factors of 10-deacetylbaccatin III, IS, DTX, and Polysorbate 80.
10-deacetylbaccatin III, it was considered possible that deacetylbaccatin III appears in the process of evaluating the stability of the formulations. However, as shown in Fig. 2(A), no peak corresponding to 10-deacetylbaccatin III was found. In addition, with the mobile phase solvent established by the present authors, the peak purities of highly hydrophobic DTX and Polysorbate 80 were both approximately 1, and symmetry was confirmed from the tailing factors, suggesting that they were reliably separated.

1. Residual Rate of GE-non-alc-DTX

Figure 3 shows the time-courses of the percentage of remaining DTX (mean ± S.D.) over 72 h after dissolving GE-non-alc-DTX in PSS (×) or 5% Glu (●). When GE-non-alc-DTX was dissolved in PSS, the percentage of remaining DTX showed no significant change over 72 h as 100.00 ± 0.75%, 101.30 ± 0.62%, 99.11 ± 0.70%, 99.30 ± 0.78%, 101.66 ± 14.28%, and 100.17 ± 0.57% after 0, 4, 12, 24, 48, and 72 h, respectively. When the solvent was 5% Glu, the percentage of remaining DTX after 72 h was 95.36 ± 3.38%, and there was no major decrease that was found when dissolved in PSS. In addition, as with separation of GE-non-alc-DTX, no significant change in the proportion of Polysorbate 80 remaining was found, being approximately 100% throughout the time-course (data not shown). No physicochemical changes such as precipitation or turbidity were observed in the glass containers in which the solutions were stored.

2. Residual Rate of OR-non-alc-DTX

Figure 4 shows the time-courses of the remaining percentage of DTX (mean ± S.D.) over 72 h after dissolving OR-non-alc-DTX in PSS (×) or 5% Glu (●). When OR-non-alc-DTX was dissolved in PSS, the proportion remaining showed a gradual decrease, being 90.92 ± 1.31% after 48 h, and it decreased significantly, to 64.72 ± 0.41%, after 72 h. On the other hand, when the solvent was 5% Glu, the percentage of remaining DTX after 72 h was 95.36 ± 3.38%, and there was no major decrease that was found when dissolved in PSS. In addition, as with separation of GE-non-alc-DTX, no significant change in the proportion of Polysorbate 80 remaining was found, being approximately 100% throughout the time-course (data not shown). No physicochemical changes such as precipitation or turbidity were observed in the glass containers in which the solutions were stored.

3. Residual Rate of STD-DTX Trihydrate and STD-DTX Anhydrate

Since OR-DTX and GE-non-alc-DTX contains DTX trihydrate and DTX anhydrate, respectively, we compared the stability of DTX trihydrate and DTX anhydrate in a solution. Figure 5 shows the time-courses of the proportion of DTX remaining (%) over 72 h after dissolving STD-DTX trihydrate (×) and STD-DTX anhydrate (●) in alcohol. In the case of the standard substances, no significant changes in the proportions of DTX remaining were found for either STD-DTX trihydrate or STD-DTX anhydrate, as they both remained consistent at approximately 100%. No physicochemical changes such as precipitation or turbidity were observed in the glass containers in which the solutions were stored.
Micelle Diameters in Formulations  Effect of diluted solution on size of micelle was investigated. Figure 6 shows the particle diameter distributions obtained by measurement of dynamic light scattering 72 h after OR-non-alc-DTX and GE-non-alc-DTX were dissolved in PSS or 5% Glu. Since the critical micelle concentration for Polysorbate 80 has been reported to be 12 μmol/L \(^5\) and the present study was carried out at concentrations sufficiently higher than that, it is considered that micelles were formed in solution. With both substances the peak was at approximately 10 nm, suggesting the presence of micelles of assembled Polysorbate 80 with both formulations.

Cloud Points To investigate stability of micelle in diluted solution, we measured cloud point that is related to the hydration of micelle. Figure 7 shows the cloud points when Polysorbate 80 and polyethylene glycol 400 were added to PSS (■) or 5% Glu (▲), and the citric acid concentration was adjusted. In a previous report,\(^6\) the cloud point decreased when sodium chloride was added to 2% polysorbate solution, and the decrease tended to be more marked when the sodium chloride concentration was greater. It was expected that in the present study the cloud point would be increased or decreased by addition of citric acid.

The cloud points were 75.4 ± 0.4°C and 83.5 ± 0.1°C when Polysorbate 80 alone was dissolved in PSS or 5% Glu, respectively, whereas they increased to 85.0...
Fig. 6. Size Distribution of DTX-micelles Determined by DLS
GE-non-alc-DTX diluted to 2 mg/mL with 5% Glu (A) or with PSS (B) and OR-DTX dissolved with 5% Glu (C) or PSS (D).

Fig. 7. Influence of Citric Acid on the Cloud Point of Polysorbate 80 and Polyethylene Glycol 400 in Various Solutions
Solutions are PSS + polysorbate 80 + polyethylene glycol 400 (■) or 5% Glu + polysorbate 80 + polyethylene glycol 400 (▲). Results are expressed as mean ± S.D., n=3.
± 0.9°C and 78.0 ± 0.8°C when polyethylene glycol 400 was added. In addition, the cloud point with PSS was lower than with 5% Glu even when citric acid was added with solutions of any concentration. In addition, the cloud point increased when citric acid was added with both PSS and 5% Glu. However, the magnitude of the increase tended to decline with increasing citric acid concentration.

**DISCUSSION**

In this study, we modified the HPLC mobile phase solvent from that specified in the pre-revision Interview Form (released October, 2013, 12th edition) for each formulation to obtain a new mobile phase solvent with higher separation performance, and evaluated DTX and impurities contained in both formulations using the improved mobile phase solvent. Neither characteristic peaks for each formulation nor peaks for degradation products were found. However, the DTX concentration showed a marked decrease after diluting OR-non-alc-DTX with PSS and leaving to stand for 24 h. In addition, in solutions prepared by dissolving STD-DTX trihydrate and STD-DTX anhydrate in alcohol, a peak was found after approximately 20 min for an unknown substance that was considered to be an impurity related to the active component.

According to the OR-DTX Interview Form (12th edition) from before the revision in October 2016, the composition of the mobile phase for the ODS solid phase contains water, methanol, and acetonitrile in a 21 : 16 : 13 ratio, and the isocratic elution method was used. According to the Interview Form (13th edition), the mobile phase should be in accordance with the Japanese Pharmacopoeia, and, in the latest version of the Japanese Pharmacopoeia (17th revised version), the method was changed to the gradient elution method. Under the previous conditions, although simple peaks could be shown, separation of Polysorbate 80 and other components was not possible, and more highly hydrophobic substances were not eluted. When the peak purity curve for the DTX absorption spectrum was measured under the actual conditions pertaining before revision, the purity deviated considerably from 1, suggesting that impurities, that is, substances other than DTX, were present. The stepwise elution method involving 1,2-dichloroethane was therefore used. As DTX is very sparingly soluble, it was essential for both the first and second mobile phase solvents to contain acetonitrile. In addition, as previous reports recommend that separation should be after adjustment of the mobile phase solvent to pH 2.5 to 4.5 using organic acids in order to avoid ester hydrolysis of DTX, an acetic acid buffer at pH 4.0 was selected in the present study. Furthermore, in order to avoid formation of other new substances due to heat load, the study was conducted with the temperature set at 30°C.

In the HPLC experiments, the concentration of each formulation was approximately five times the concentration of 0.4 mg/mL used in clinical practice. This is because the peak area was accurately measured at a concentration of 2.0 mg/mL among concentrations of 0.004 to 4.0 mg/mL, and the reduction of changes with time shown by HPLC was avoided at this concentration. In addition, it has been reported that DTX was administered inside the bladder in clinical practice and the final concentration is adjusted to 0.75 mg/mL using PSS. Although the content of the active component was lower and the content of impurities was higher in the GE-DTX formulation in a report comparing the active component of and impurities in the OR-DTX formulation with the commercially available GE-DTX formulation overseas, no such peaks were observed but in the present study. Probably, the contents of impurities in GE-DTX formulations that have been launched by pharmaceutical companies in Japan are lower than those in GE-DTX formulations overseas.

Because the polyethylene glycol 400 contained in the DTX formulation does not show specific UV absorption, the elution time for the mobile phase solvent used is unknown. However it is at least considered that there was no superposition of the peak with polyethylene glycol 400 on the basis of the DTX peak purity test. In addition, whereas the DTX formulation dissolves after forming micelles with the nonionic surface-active agent Polysorbate 80, as the solvent used was ethanol for standard substances DTX trihydrate and DTX anhydrate, it was considered that they dissolve in the form of molecular state. The separation profiles obtained in the present study show that the formulation and standard substance are both eluted at an elution time of 13 min. This suggests that the DTX micelles present in each formulation are broken down by organic solvents in the mobile phase, resulting in the same elution time as for DTX.
All the concentrations of DTX in these formulations were higher than the aqueous solubility of DTX (4.93 μg/mL), and it is considered that Polysorbate 80, included in the formulations as an excipient, forms micelles to increase the solubility of DTX. In this context, micelle diameter was measured by the dynamic light scattering method in order to investigate the differences in micelle forms between the formulations. The micelle diameter in each formulation was approximately 10 nm, but the diameter in GE-non-alc-DTX, which contains polyethylene glycol 400 and anhydrous citric acid, tended to be greater than that in OR-non-alc-DTX. It is suggested that this difference is due to the loosened the micelle packing by the polyethylene glycol 400 contained in GE-non-alc-DTX. In addition, although both formulations tended to be larger in PSS than in 5% Glu, it is considered that micelle size did not have a very marked effect in general.

According to the International Pharmaceutical Excipients Council Japan (http://www.jspc.or.jp), citric acid has various uses, as a stabilizer, buffer, antioxidant, toxicity-adjusting agent, pH-adjusting agent, filler, dispersant, anti-decay agent, preservative, solubilizing agent, and insecticide. Among these, its antioxidant effect is strongest, and, making use of this, it is in current use as a pharmaceutical additive in situations in which stabilization of the product is essential. There were no changes in residual DTX in GE-non-alc-DTX with time up to 72 h with either PSS or 5% Glu (Fig. 3). Interactions between the citric acid carboxyl group and the cations in PSS, and/or chelation by citric acid and cations may reduce the salt concentration, and can thus in turn reduce the salting-out effect. In addition, interactions are formed between the carboxyl groups of citric acid and the polyoxyethylene groups that function as the hydroxyl groups of polyethylene glycol 400, and it is suggested that the resulting negative charges on micelles increase stability. It is therefore considered that inclusion of polyethylene glycol 400 and anhydrous citric acid in GE-non-alc-DTX increases DTX stability. When OR-non-alc-DTX was dissolved in PSS, significant changes with time were found from 24 h (Fig. 4). The DTX present in the solvent in the form of a hydrophilic colloid is in unstable despite being solvated, and it is suggested that the presence of electrolytes thus leads to salting out. The non-micellization of polyethylene glycol 400 and anhydrous citric acid is another factor in this, suggesting that micellization of these substances may contribute to DTX stability.

When the cloud points were measured after adding Polysorbate 80 and polyethylene glycol 400 to PSS or 5% Glu, the PSS cloud point was lower than the 5% Glu cloud point (Fig. 7). In comparison with 5% Glu, PSS readily induces the salting out of Polysorbate 80, which is a surfactant, and it is considered that micelle stability by Polysorbate 80 is reduced. In addition, analysis of effects of polyethylene glycol 400 at the same quantity with the Polysorbate 80, which is only contained in GE-non-alc-DTX, and an appropriate quantity of citric acid, showed that the cloud point increased with addition of polyethylene glycol 400 and with increasing citric acid concentration. It is therefore considered that addition of polyethylene glycol 400 and citric acid reduces the salting-out effects of Polysorbate 80. This effect was particularly marked with PSS. It is therefore considered that, when OR-non-alc-DTX was dissolved in PSS, Polysorbate 80 would be subject to salting-out effects more readily than in the other cases, resulting in decreased Polysorbate 80 solubility, and thus precipitation.

In the present study, when OR-non-alc-DTX was diluted in PSS, the DTX peak area, with a retention time of 13 min, started to decrease from approximately 24 h after dilution and decreased to 90.92% and 64.72% after 48 and 72 h, respectively. At the same time white floating substance was observed, and this increased with time (Fig. 4). Because no peak areas other than that of DTX decreased and no new peaks appeared, it is considered that this floating substance was derived from DTX itself. At the present time, the identity of the precipitated white material is uncertain. This finding clearly shows that administration must be completed within 24 h after dilution if OR-non-alc-DTX is diluted in PSS in clinical practice. In fact, Eroles et al. have reported that, if DTX is diluted in PSS, its stability decreases when more than 24 h has passed, leading to decreased concentration. In the present study, it was also clearly shown that if DTX is diluted in PSS its stability decreases with time from 24 h after dilution, leading to concentration decrease. On the other hand, it has been reported that the stability of a solution in PSS at 23°C is such that at least 95% remains after 35 days, and Thiesen has reported that DTX stability was favorable for at least 5 days when OR-non-alc-
DTX not containing either polyethylene glycol 400 or anhydrous citric acid was diluted in PSS or 5% Glu.\(^{19}\)

The reason why the present results are different from those reported previously is unknown. It is possible that the storage conditions, such as temperature and additives, may have affected the stability.

The DTX concentration did not decrease when OR-non-alc-DTX was diluted in 5% Glu. In addition, the same was the case when GE-non-alc-DTX was diluted in PSS or 5% Glu, and when STD-DTX trihydrate or STD-DTX anhydrate was diluted in ethanol (Figs. 3 and 5). On the basis of the above results, it was considered that OR-DTX is a colloidal dispersion and that as the colloidal state becomes unstable in the presence of electrolytes such as PSS, resulting in flocculation. However, as no such phenomenon has been observed with OR-non-alc-DTX, it is considered that the floating material must have been precipitated by a different mechanism.

The differences between the characteristics of OR-DTX and GE-non-alc-DTX are shown in Table 1. Although OR-DTX includes Polysorbate 80 as an additive in addition to the active component, the content is described as appropriate, the actual content is uncertain. On the other hand, GE-non-alc-DTX includes polyethylene glycol 400 as well as Polysorbate 80 and anhydrous citric acid as a pH-stabilizer, and the potential for these to modify stability cannot be ruled out. However, possible effects of anhydrous citric acid, which is not included in OR-DTX as an additive, cannot be ruled out. As explained above, DTX is a key drug for treating various cancer types, and it is therefore considered necessary to clarify whether the differences in chemical behavior between the original and generic formulations have major effects on efficacy and safety. The most important benefit of the switch from the original formulation to a generic formulation is decrease in cost. However, since generic formulations are evaluated on the basis of data from bioequivalence studies, elution studies, and stability studies, little information is available about clinical use. Bioequivalence studies, albeit only a small number, have been conducted with letrozole, and comparison of the original and generic drugs showed no differences in efficacy or safety.\(^{20-22}\) On the other hand, a quality-related comparison of original and generic drugs was reported in a study by HPLC when adverse effects were found after a generic formulation of ritodrine hydrochloride for injection was used.\(^{23}\) In that study, the number and quantities of impurities contained were higher in the generic than the original formulation, and the quantities of impurities contained also showed considerable variation between lots of the generic formulation. There have also been similar reports of research on nafamostat mesylate,\(^{24}\) and several reports of adverse effects possibly due to impurities that are related to the active component. In some generic formulations, such as the formulation studied in the present study, there are differences in the composition and content of the additives that are included in the original formulation, and there is, therefore, the potential for unpredicted adverse effects. For example, in a comparative investigation of renal dysfunction caused by original and generic cisplatin formulations, the generic formulation caused dysfunction more readily, and also caused slightly more severe dysfunction.\(^{25,26}\)

The rate of spread of generic drugs in Japan tends to be low in comparison with Europe and the USA,\(^{27,28}\) although the cost of anti-cancer agents, in particular, is high relative to drugs used in other fields among prescription drugs. Reasons that have been suggested for this include uncertainties about quality, uncertainties about equivalence in clinical practice, and the limited information provided about the products in generic drugs. It has been reported that it is essential to select therapeutic agents with high cost-performance by demonstrating no differences between the formulations in terms of patient’s quality of life as well as efficacy and adverse events.\(^{29}\) Therefore, for establishment of the reliability of generic drugs, it is important to follow the approach taken in the present study, starting with de novo establishment of the mobile phase in order to increase the separation performance of the original and generic formulations, and then to evaluate clinical safety, conduct purity tests, and ascertain the decomposition rate. In the present study, these items were verified, with the resulting data providing a useful basis for transfer from the original drug to the generic drug, and it is hoped that they will make a significant contribution in clinical practice.

**CONCLUSIONS**

In the present study, when OR-non-alc-DTX was diluted in PSS, the DTX concentration showed a linear decrease from 24 h after dilution, and the effect
was marked from 48 h after dilution. In the case of dilution in 5% Glu, the concentration decrease was not comparable to that with PSS. On the other hand, in the case of GE-non-alc-DTX, no DTX concentration decrease with time was found, and the formulation was stable. On the basis of these findings, it is recommended that DTX be used within 24 h after dilution. In addition, since major effects were found when OR-non-alc-DTX was diluted in PSS, it should be borne in mind that there are differences that depend on the diluent solvent, and it is considered that 5% Glu should be used as the diluent solvent until the mechanisms of these differences have been elucidated.

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Conflict of Interests The authors have no potential COI to disclose.

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