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Stability indicating RP-HPLC method development for docetaxel trihydrate

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ABSTRACT

The purpose of present study was to develop RP-HPLC method for estimation of docetaxel trihydrate which should be a suitable, simple, precise, accurate, robust, and reproducible. The samples were assayed by the Shimadzu HPLC instrument - LC-20AD (Japan) equipped with Shimadzu SPD-M20A UV-VIS detector operated at wavelength of 230 nm. The binary gradiant pump was used for the analytical method development. The reverse phase stainless steel column (150 × 4.6 mm) packed with 5 µm particles (C-8, LiCrosphore® 100, Germany) was used to take chromatograph. A mobile phase consisting of acetonitrile/phosphate buffer 20 mM, (45:55, v/v), pH 3.5 adjusted with o-phosphoric acid at a flow rate of 1 mL/min. The method was validated by system suitability and reproducibility. The linearity was also determined using samples with five different concentrations of 20, 40, 60, 80 and 100 µg/mL. The results of the study showed that the developed RP-HPLC method is simple and robust which is useful for the estimation of docetaxel trihydrate in bulk drug and in pharmaceutical dosage form. The results of stability show that the method has stability over a period of 48 h at room temperature.

Keyword: Docetxel trihydrate, RP-HPLC method, validation, stability

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1. INTRODUCTION

Docetaxel [4-acetoxy-2α-benzoyloxy-5β,20-epoxy-1,7β,10β-trihydroxy-9-oxotax-11-ene-11α-yl-(2R,3S)-3-tert-butoxy-carbonylamino-2-hydroxy-3-phenylpropionate] (see **Figure 1**) is a semisynthetic antineoplastic agent which is derived from a natural inactive precursor 10-deacetyl baccatin III. This was extracted from leaves of Taxus baccata **[1, 2].** Docetaxel is widely used chemotherapeutic agents especially in the breast cancer therapy **[3]** and it was approved by the United States Food and Drug Administration (USFDA) for the treatment of castration resistant prostate cancer (CRPC) **[4].** It belongs to BCS Class II with practical insolubility in water (4.93 µg/mL) **[5]** and high permeability. It has a λmax of 230 nm and hence it possesses very poor oral bioavailability **[6].** It acts by blocking the cellular mitotic and interphase functions **[7, 8]** and its anticancer activity is dependent on its concentration and duration of exposure **[9, 10].** Their cell specific activity and various targeted drug delivery were prepared for enhancing their efficacy and site specific action **[11].** The pharmacokinetic and tissue distribution studies for DTX–loaded micelles shows significantly higher retention concentration in plasma and tumor tissue compared with free DTX **[12, 13].**

The present study was aimed to develop a simple, rapid and robust RP-HPLC method for quantification of DTX. The method was validated based on parameters like system suitability, specificity, robustness. The stability of developed method was also studied at room temperature.

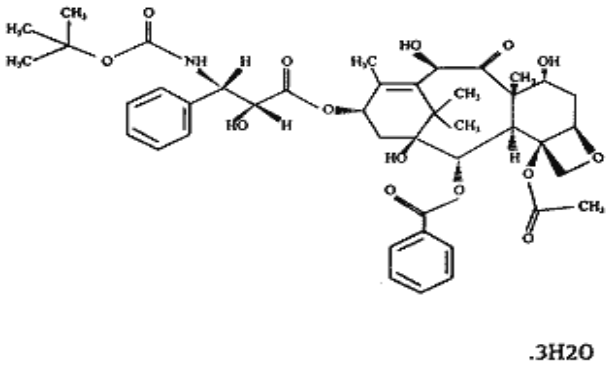

Figure 1: Chemical Structure of docetaxel trihydrate

2. MATERIALS AND METHOD

2.1 Materials

Docetaxel trihydrate (DTX) was obtained as a gift sample from Scino Pharmaceutical Pvt., Taiwan. HPLC grade acetonitrile was purchased from Thermo Fisher Scientific India Pvt. Ltd. o-phosphoric acid was purchase from Loba Chemi Pvt. Ltd. (India). Water was purified on a Milli-Q system obtained from a Millipore® synergy system (Millipore, Billerica, Massachusetts, USA). All other chemicals used were of analytical grade.

2.2 Instrument and chromatographic condition

A Shimadzu HPLC instrument - LC-20AD (Japan) equipped with Shimadzu SPD-M20A UV-VIS detector operated at wavelength of 230 nm and binary gradiant pump was used for the analytical method development. The reverse phase stainless steel column (150 × 4.6 mm) packed with 5 µm particles (C-8, LiCrosphore® 100, Germany) was used to take chromatograph.

2.3 Preparation of mobile phase and drug sample

2.3.1 Preparation of buffer for mobile phase

Phosphate buffer (20 mM) pH 3.5 was prepared by dissolving 1.31g of sodium dihydrogenphosphate and 1.05 g of phosphoric acid in HPLC water. Adjust the pH 3.5 using o-phosphoric acid. Filter the solution under reduced pressure to remove insoluble substance using 0.45 µm filters.

2.3.2 Preparation of mobile phase

A mobile phase containing acetonitrile and phosphate buffer (45:55, v/v) was prepared with 45% v/v acetonitrile and 55% v/v of phosphate buffer 20 mM, pH 3.5 adjusted with o-phosphoric acid. Sonicate for 15 min and filter through 0.45 µm filter before use.

2.3.3 Preparation of standard stock solution

A stock solution of DTX was prepared by dissolving it in HPLC grade acetonitrile. Weighed accurately about 10 mg of DTX was transferred to the 10 mL volumetric flask and dissolve in HPLC grade acetonitrile. The volume was made upto 10 mL to get final concentration of 1000µg/mL for analyte.

2.3.4 Preparation of sample

A stock solution of DTX (1000 µg/mL) was used for subsequent dilutions to obtained five concentrations 20, 40, 60, 80 and 100 µg/mL. The samples were appropriately diluted with the acetonitrile to obtained desired concentrations and filtered through a 0.22 µm pore size filter before injection.

2.4 Method development

DTX concentration was measured using HPLC analysis (SPD-M20A Shimadzu pump, LC-20AD Shimadzu UV-vis detector) at 230 nm. The samples were chromatographed on a 150 × 4.6 mm reverse phase stainless steel column packed with 5 µm particles (C-8, LiCrosphore® 100, Germany) and eluted with a mobile phase consisting of acetonitrile/phosphate buffer 20 mM, (45:55, v/v), pH 3.5 adjusted with o-phosphoric acid at a flow rate of 1 mL/min. The column temperature was maintained at 25 ºC. The samples were appropriately diluted with acetonitrile and injected (20 µL) directly into the injector of HPLC system with the help of syringe without further treatment.

2.5 Validation of the RP-HPLC method

2.5.1 System suitability

The system suitability was assured by determining peak retention time, peak area, theoretical plates and tailing factor for DTX. The prescribed values for system suitability are CV < 1%, asymmetry factor < 2 and theoretical plates >2000 **[14].** The standard concentration of 1000 µg/mL was used in the preparation of the samples. The sample preparation was done according to the method described in **Section 2.3**; therefore, the final nominal concentration, to be detected by the described method, was 20 µg/mL. The samples were assayed for determining the system suitability in replicates of six [14].

2.5.2 Linearity

The calibration curve for DTX was prepared in mobile phase consisting of acetonitrile/phosphate buffer 20 mM, (45:55, v/v) having pH 3.5 with concentrations of 20, 40, 60, 80 and 100 µg/mL at a wavelength of 230 nm. Five standard concentrations of 20, 40, 60, 80 and 100 µg/mL were used in the linearity study of samples of DTX. All the samples were prepared according to the method as described above in **Section 2.3.** The slope, intercept and correlation coefficient (r^2) were calculated for regression analysis of DTX. As per FDA guidelines 15% deviation from the nominal concentrations is permissible, except at LLOQ deviation should not be more than 20%.

2.6. Stability

The standard with 100 µg/mL concentration was used in the preparation of samples for testing the stability. The samples were prepared in accordance with the method as described above in Section 2.3; therefore, the final nominal concentration was 20 µg/mL. The stability of DTX in the prepared sample was determined by analysing concentration at 1, 6, 12, 24 and 48 h. The concentration was determined for DTX at each time point (n = 3).

3. RESULT AND DISCUSSION

HPLC method development was done in mobile phase consisting of acetonitrile/phosphate buffer 20 mM, (45:55, v/v), pH 3.5 adjusted with o-phosphoric acid, with this mobile phase satisfactory resolution between solvent and drug peaks was obtained. The flow rate of 1 mL/min was selected because of its ideal retention time, proper resolution and lesser time for analysis. Under these chromatographic conditions the elution of docetaxel trihydrate was observed to be optimum. The drug was eluted at retention time (RT) 4.6 with peak intensity 45 at 230 nm with peak tailing about 2.05 ± 0.55 which confirms the system suitability, as shown in **Figure 2.**

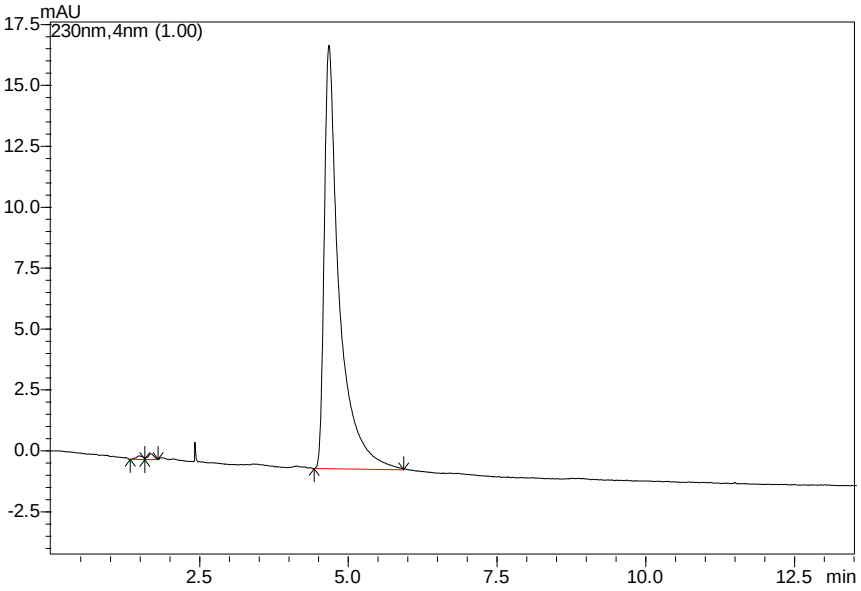

Figure 2: Standard chromatogram of docetaxel trihydrate

Table 1: System Suitability for docetaxel trihydrate method development

Ret. Time	Theoretical Plates	Peak Area	Height	Resolution	Tailing Factor
4.668	2280.409	306008	17379	10.069	2.502

The detector response was found to be linear in the range. Using the MS Excel the trendline was plotted, with the regression line equation (slope and intercept) and data analyzed. The areas under curve values obtained are shown in **Table 2**. The calibration of the peak area against concentration of DTX was found to be y=11226x+15282 with r^2 = 0.993 for the DTX concentrations of 20, 40, 60, 80 and 100 µg/mL (where y: peak area and x: DTX concentration) as shown in **Figure 3**, the detector response was linear in the concentration of 0-100 µg/mL and the limit of detection was found to be 0.03 µg/mL.

Table 2: Area under curve data of linearity of response for docetaxel trihydrate

Sr. No.	Concentration (µg/mL)	AUC
01	20	283425
02	40	355648
03	60	483421
04	80	606008
05	100	719551

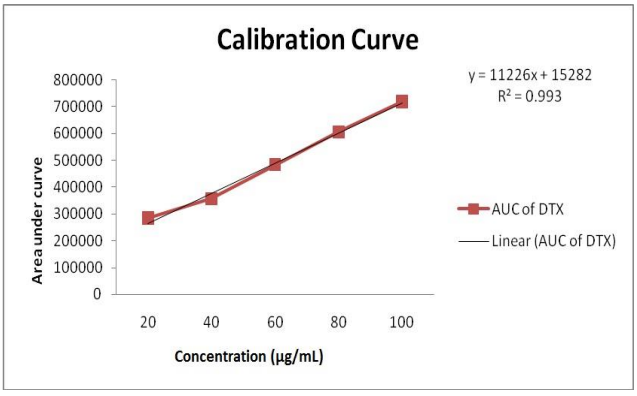

Figure 3: Calibration curve for DTX by HPLC method

The stability of developed RP-HPLC method was performed at room temperature for period of 48 h. The results of concentrations obtained on various time point of 1, 6, 12, 24 and 48 shows that there was no significant difference in concentrations at various time point. Hence the developed method was found to be suitable and having stability at room temperature.

4. CONCLUSION

The present RP-HPLC method for quantification of DTX proved to be simple, robust and reproducible as per the FDA guidelines. The analyte was found to be stable in the samples kept at room temperature for 48 h. Hence the developed method can be used for estimation in various dosage form and analysis of DTX.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

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