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Estimation of Docetaxel in Parenterals by RP-HPLC

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A simple, precise, rapid and accurate reverse phase HPLC method developed for the estimation of docetaxel in parenterals dosage form. A Unison YMC C_{18}, 250 mm × 4.6 mm i.d., 5 µm particle size, with mobile phase consisting of acetonitrile and 0.02 M ammonium acetate in water (pH 4.5 adjusted with orthophosphoric acid) in the ratio of 45:55 v/v was used. The flow rate was 1.5 mL/min and the effluents were monitored at 230 nm. The retention time was 5.82 min. The detector response was linear in the concentration of 1-200 mcg/mL. The respective linear regression equation being Y = 46487x - 36391. The limit of detection and limit of quantification was 0.03 and 0.09 mcg/mL, respectively. The percentage assay of docetaxel was 99.09 %. The method was validated by determining its accuracy, precision and system suitability. The results of the study showed that the proposed RP-HPLC method is simple, rapid, precise and accurate, which is useful for the routine determination of docetaxel in bulk drug and in its pharmaceutical dosage form.

Key Words: Docetaxel, RP-HPLC, Estimation, Accuracy studies, Pharmaceutical dosage and Parenterals.

INTRODUCTION

Docetaxel is a natural product with antitumor activity. Docetere (docetaxel) is obtained *via* a semi-synthetic process from *Taxus baccata*. The chemical name for docetaxel is (2R,3S)-N-carboxy-3-phenylisoserine, N-*tert*-butyl ester, 13-ester with 5,20-epoxy-1,2,4,7,10,13-hexahydroxytax-11-en-9-one-4-acetate-2-benzoate, trihydrate. Docetaxel is a white to off-white crystalline powder with the empirical formula $C_{43}H_{53}NO_{14}$ and a molecular weight of 807.8 g/mol. It is slightly soluble in water and melts at around 216-217 °C. Literature survey reveals many chromatographic methods[1-9] for the determination of docetaxel in biological fluids. So far, only one assay procedure has been reported for the estimation of docetaxel from pharmaceutical dosage forms. The availability of an HPLC method with high sensitivity and selectivity will be very useful for the determination of docetaxel in pharmaceutical formulations. The aim of the study is to develop a simple, precise and accurate reversed-phase HPLC method for the estimation of docetaxel in bulk drug samples and in pharmaceutical dosage form.

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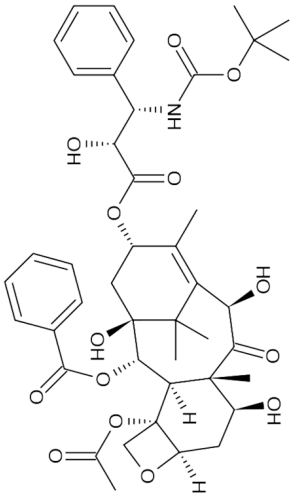

Structure of docetaxel

EXPERIMENTAL

Docetaxel was obtained as a gift sample from JNT University, Hyderabad. Ammonium acetate and orthophosphoric acid were of analytical grade and supplied by M/s S.D. Fine Chem Limited, Mumbai. Acetonitrile and water used were of HPLC grade (Qualigens). Commercially available docetaxel injection (Docetere® 16.7 mL Vial, Dr. Reddy's Laboratories) was procured from local market.

Quantitative HPLC was performed on liquid chromatograph, waters separation 2996, PDA detector module equipped with automatic injector with injection volume 20 µL and 2693 pump. A Unison YMC C_{18} column (250 mm × 4.6 mm i.d., particle size 5 µm) was used. The HPLC system was equipped with Empower Software.

HPLC conditions: The contents of the mobile phase were acetonitrile and 0.02 M ammonium acetate in water (pH 4.5 adjusted with orthophosphoric acid) in the ratio of 45:55 v/v was used. They were filtered before use through a 0.45 µm membrane filter and pumped from the respective solvent reservoirs to the column at a flow rate of 1.5 mL/min. The run time was set at 15 min and the column temperature was ambient. Prior to the injection of the drug solution, the column was equilibrated for at least 0.5 h with the mobile phase flowing through the system. The eluents were monitored at 230 nm.

Preparation of standard stock solution: A standard stock solution of the drug was prepared by dissolving 10 mg of docetaxel in 10 mL volumetric flask containing 5 mL of diluent (50:50 v/v acetonitrile:water), sonicated for about 15 min and then made up to 10 mL with diluent to get 1 mg/mL standard stock solution.

Working standard solution: 1 mL of the above stock solution was taken in 10 mL volumetric flask and thereafter made up to 10 mL with diluent (50:50 v/v acetonitrile:water) to get a concentration of 100 µg/mL.

Preparation of sample solution: Docetere® (docetaxel) injection is a clear, colourless to slightly yellow viscous solution. It is supplied as a nonaqueous solution intended for dilution with a suitable parenteral fluid prior to intravenous infusion. Docetere is available in 30 mg (5 mL), 100 mg (16.7 mL) and 300 mg (50 mL) multidose vials. Each mL of sterile nonpyrogenic solution contains 6 mg docetaxel, 527 mg of purified Cremophor® EL (polyoxyethylated castor oil) and 49.7 % (v/v) dehydrated alcohol, USP. Accurately pipette out 3 mL of docetaxel [equivalent to 30 mg of docetaxel from the contents of the three vials (Docetere® 5 mL vial, Dr. Reddy's Laboratories) [each 5mL vial containing 30 mg of docetaxel] and transferred in to 10 mL volumetric flask containing 5 mL of diluent (50:50 v/v acetonitrile:water). The mixture was allowed to stand for 0.5 h with intermittent sonication to ensure complete solubility of the drug and then filtered through a 0.45 μm membrane filter, followed by adding diluent to obtain a stock solution of 1 mg/mL. An aliquot the 1 mL of this solution was transferred to a 10 mL volumetric flask and made up to sufficient volume with diluent (50:50 v/v acetonitrile:water) to give an concentration of docetaxel sample solution of 100 mcg/mL.

Linearity: Aliquots of standard docetaxel stock solution were taken in different 10 mL volumetric flasks and diluted up to the mark with the diluent such that the final concentrations of docetaxel are in the range of 1-200 mcg/mL Each of these drug solutions (20 μL) was injected three time into the column and the peak area and retention time were recorded. Evaluation was performed with PDA detector at 230 nm and a calibration graph was obtained by plotting peak area *versus* concentration of docetaxel (Fig. 1). The plot of peak area of each sample against respective concentration of docetaxel was found to be linear in the range of 1-200 μg/mL with correlation coefficient of 0.9999. Linear regression least square fit data obtained from the measurements are given in Table-1. The respective linear regression equation being Y = 46487.83x - 36391. The regression characteristics, such as slope, intercept and RSD % were calculated for this method and given in Table-1.

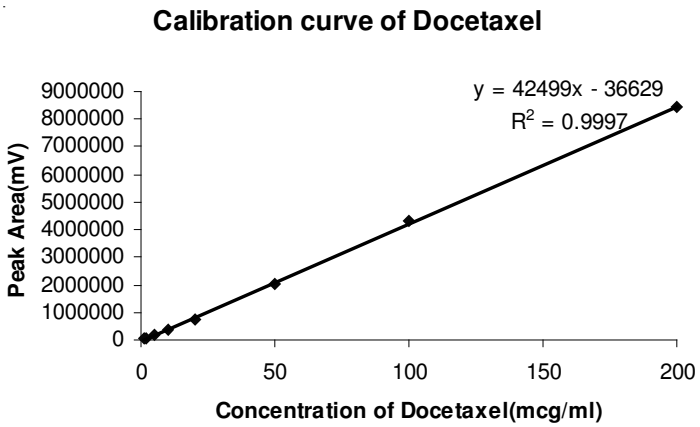

Fig. 1. Calibration curve of docetaxel by RP-HPLC

TABLE-1
LINEAR REGRESSION DATA FOR CALIBRATION CURVES

Drug	Docetaxel
Concentration range (mcg/mL)	1-200
Slope (m)	46487
Intercept (b)	36391
Correlation coefficient	0.9997
RSD (%)	0.18

Assay: 20 µL of sample solution was injected into the injector of liquid chromatograph. The retention time was found to be 5.8 min. The amount of drug present per vial was calculated by comparing the peak area of the sample solution with that of the standard solution. The data are presented in Table-2.

TABLE-2
RESULTS OF HPLC ASSAY

Concentration taken (mcg/mL)	Mean ± SD (n = 6)	CV (%)
10	10.02 ± 0.138	1.38
5	5.02 ± 0.143	2.86
2	2.03 ± 0.067	3.32

Average of three different concentration levels.

Recovery studies: Accuracy was determined by recovery studies of docetaxel, known amount of standard was added to the pre-analyzed sample and subjected to the proposed HPLC analysis. Results of recovery study are shown in Table-3. The study was done at three different concentration levels.

TABLE-3
RESULTS OF ACCURACY STUDY

Amount of drug added (mcg) to 10 mcg	Mean ± SD amount (mcg) recovered (n = 3)	Mean ± SD % recovery
10	10.12 ± 0.14	101.2 ± 1.98
5	4.95 ± 0.22	99.00 ± 2.54
2	1.97 ± 0.31	98.5 ± 4.21

Average of three different concentration levels.

RESULTS AND DISCUSSION

The system suitability tests were carried out on freshly prepared standard stock solution of docetaxel. Parameters that were studied to evaluate the suitability of the system are given in Table-4.

Limit of detection (LOD) and limit of quantification (LOQ): The limit of detection (LOD) and limit of quantification (LOQ) for docetaxel were found to be 0.03 and 0.09 µg/mL, respectively. The signal to noise ratio is 3 for LOD and 10 for LOQ. From the typical chromatogram of docetaxel as shown in Fig. 2, it was found

TABLE-4
VALIDATION SUMMARY

Validation parameter		Results
System suitability	Theoretical plates (N)	2142
	Tailing factor	1.57
	Retention time (min)	5.8
LOD (mcg/mL)		0.03
LOQ (mcg/mL)		0.09

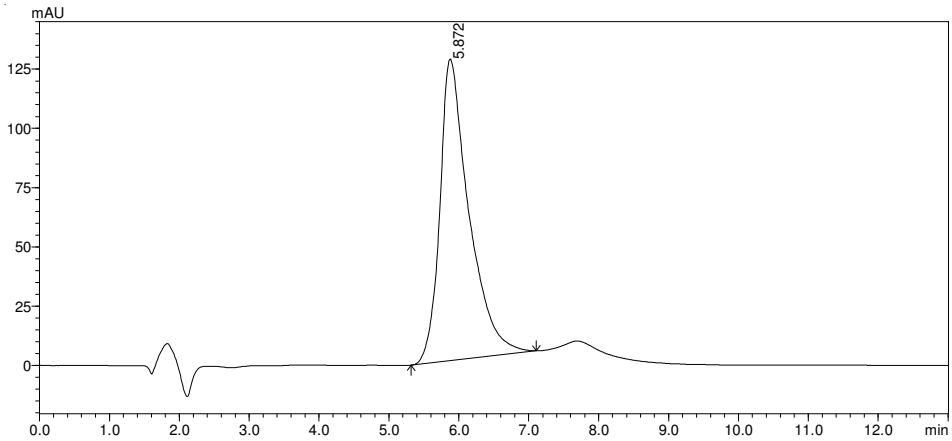

Fig. 2. Typical chromatogram of docetaxel by RP-HPLC

that the retention time was 5.821 min. A mixture of acetonitrile and 0.02 M ammonium acetate in water (pH 2.5 adjusted with orthophosphoric acid) in the ratio of 45:55 v/v was found to be most suitable to obtain a peak well defined and free from tailing. In the present developed HPLC method, the standard and sample preparation required less time and no tedious extraction were involved. A good linear relationship (r = 0.9999) was observed between the concentration range of 1-200 mcg/mL. Low values of standard deviation are indicative of the high precision of the method. The assay of docetaxel parenteralss was found to be 99.69 %. From the recovery studies it was found that *ca.* 101.25 % of docetaxel was recovered which indicates high accuracy of the method. The absence of additional peaks in the chromatogram indicates non-interference of the common excipients used in the parenteralss. This demonstrates that the developed HPLC method is simple, linear, accurate, sensitive and reproducible.

Thus, the developed method can be easily used for the routine quality control of bulk and parenterals dosage form of docetaxel within a short analysis time.

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