3.1 Estimation of Absorption Maxima Of Docetaxel

Docetaxel is a semi-synthetic drug, belonging to taxoid family, derived from precursor extracted from the renewable needle biomass of the European yew tree, Taxus baccata. Docetaxel, which is chemically 1,7β,10β-trihydroxy-9-oxo-5β,20-epoxytax-11-ene-2α,4,13α-triyl 4-acetate 2-benzoate 13-{(2R,3S)-3-[(tert-butoxycarbonyl)amino]-2-hydroxy-3-phenylpropanoate} trihydrate, an anti-neoplastic agent most commonly used in the treatment of breast, ovarian, prostate, and non-small cell lung cancer.(1–3) The molecular empirical formula for Docetaxel is $C_{43}H_{53}NO_{14}.3H_2O$. The structure of Docetaxel is shown in Figure 3.1.

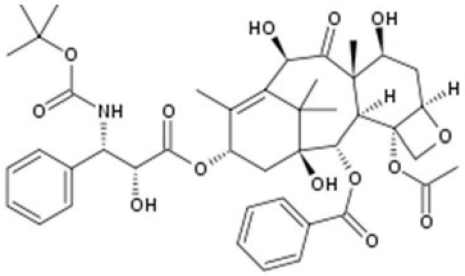

Figure 3.1 Structure of Docetaxel

It is highly lipophilic and practically insoluble in water, soluble in ethanol, methanol, chloroform, acetone, acetonitrile.(4)

It is available in the market as an injection to be administered intravenously. Docetaxel is official in USP.(3) The methods of estimation of Docetaxel in bulk drug and the formulation include the chromatographic methods using UV detector. Other reported methods of analysis are reverse phase and ion pair HPLC methods. So far, no Ultraviolet spectrophotometric method for estimation of Docetaxel is reported. Ultraviolet spectrophotometric methods are simpler and faster compared to the chromatographic methods. Docetaxel in acetonitrile exhibits a sharp peak at 229 nm when scanned in the UV region between 200-400 nm and hence it was selected as the analytical wave-length. Thus, the aim of the present investigation was to develop a reliable spectrophotometric procedure for estimation of docetaxel in bulk and marketed formulation.(5–10)

3.1.1 Equipment

Digital analytical balance (Mettler Toledo), Double beam UV-VIS spectrophotometer.

3.1.2 Methods

3.1.2.1 Determination λmax

The absorption maximum (λ max) of docetaxel was determined by scanning 10 µg/ml solution against Acetonitrile as reagent blank in spectrum mode between 200 to 400nm.

3.1.2.2 Preparation of Stock Solution

Primary stock solution of docetaxel in acetonitrile (1mg/10ml) was prepared by dissolving 10 mg docetaxel in 100ml acetonitrile. The primary stock solution was stored at 2-8ºC.

3.1.2.3 Preparation of Calibration Curve in Acetonitrile

Appropriate aliquots of the stock solution of docetaxel (1mg/10ml) were transferred to 10ml volumetric flasks and were diluted up to the mark with acetonitrile. The absorbance of all the prepared solutions (5, 10, 15, 20, 25, 30, 35, 40 and 45 µg/ml) was then measured at the absorbance maxima, 229nm against the reagent blank (acetonitrile) using UV-VIS spectrophotometer. The readings were recorded in triplicate. Mean value (n=3) along with the standard deviation (SD) are recorded in Table 1. The regressed values of absorption were plotted graphically against the concentrations, as shown in Figure 3.1. Stability of the solutions of docetaxel in acetonitrile was ascertained by observing the changes in the absorbance of the solution at the analytical wavelength, over a period of 24 hours, at room temperature. The readings were recorded in triplicate.

3.2 Analytical Method Validation

3.2.1 Linearity

The linearity of an analytical method is its ability to elicit, test results that are directly or well-defined mathematical transformation proportional to the concentration of analyte in samples within a given range. Beer's law states that absorbance is proportional to the concentration of the absorbing species. A calibration curve is prepared by plotting a dependent variable (absorbance Y) as a function of an independent variable (concentration X). This relation if found with a series of measurements, which in practice, is often a linear one.(11–13,8)

3.2.2 Accuracy

Accuracy of an analytical method is the closeness of the test results obtained by that method to true value. Accuracy, sometimes also referred to as recovery is an indicator of the trueness of test measurements. To determine the accuracy of the method three quality control samples (10µg/ml, 20µg/ml and 30µg/ml) for acetonitrile were used. The samples chosen were such to represent the entire range of the standard curve i.e lower, middle and higher concentrations of the range. Accuracy was calculated by analysis of 3 replicate samples for the above described methods. (11–13,8)

3.2.3 Precision

Precision of an analytical method is the degree of agreement among the individual test results when the procedure is applied repeatedly to multiple scanning of homogenous sample. Precision may be measure of either degree of reproducibility or of repeatability of the analytical method under normal operating conditions. The precision of an analytical method is usually expressed as the standard deviation or confidence limit. (1,11–13,8)

3.2.4 Limit of Detection and Limit of Quantification:

The Limit of Detection (LoD) is a quantitative parameter. It is the lowest concentration of the analyte in a sample that can be detected with acceptable precision and accuracy under stated experimental conditions. It is expressed as the concentration of the analyte in the sample. The limit is expressed in terms of µg/ml, ng/ml, pg/ml. etc. LoD values are specific for a particular set of experimental conditions. Anything that changes the sensitivity of a method, including instrument, sample preparation etc. will change the detection limits. (1,11–13,8)

3.3 Results and Discussion:

3.3.1 Determination of λ max

Based on the spectrophotometric scanning of docetaxel (10µg/ml), the maxima was obtained at 229nm in acetonitrile, hence chosen as the analytical wavelength.

3.3.2 Calibration Curve of Docetaxel

Table 1 shows the mean absorbance values along with the standard deviation of docetaxel in acetonitrile. The high correlation coefficient in the acetonitrile indicated that absorbance and concentration of the drug was linearly related. Beer's law was found to be obeyed in the range of 5 to 45µg/ml in acetonitrile.

Table 3.1 Absorbance of Docetaxel in Acetonitrile

Concentration (µg/ml)	Absorbance ± SD
5	0.114 ± 0.008
10	0.221 ± 0.013
15	0.329 ± 0.010
20	0.437 ± 0.018
25	0.545 ± 0.016
30	0.653 ± 0.020
35	0.761 ± 0.017
40	0.869 ± 0.009
45	0.977 ± 0.012

*Mean ± SD (n =3)

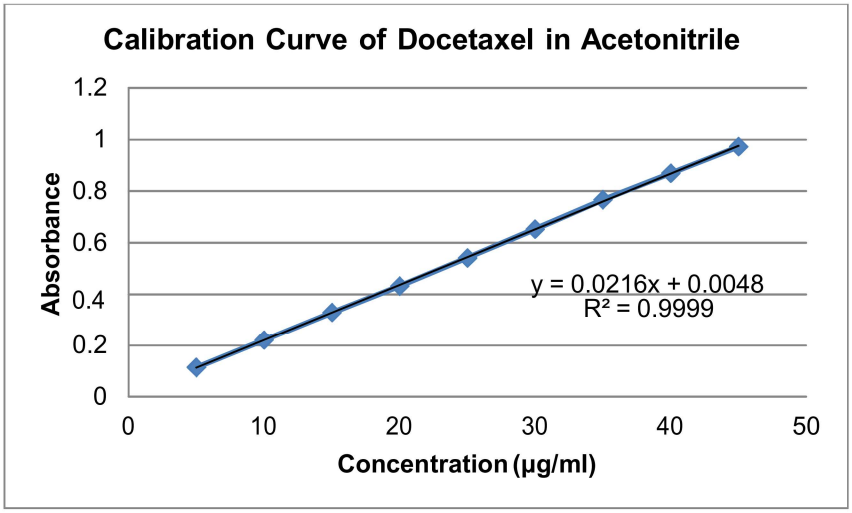

Figure 3.2 Calibration Curve of Docetaxel in Acetonitrile

3.3.3 Results of Analytical Method Validation

Linearity of the Assay

The linearity of the assay was determined by plotting the standard calibration curves for the concentration range 5 - 45 µg/ml at 229nm in acetonitrile for 3 consecutive days.

Regression Equation: $y = 0.0216x + 0.0048$, $R^2 = 0.9999$

Accuracy

The excellent mean % Accuracy values, close to 100 %, and their very low standard deviation values (SD < 1.0) represent high accuracy of the analytical methods. The mean % accuracy for lower (10 µg/ml), intermediate (20 µg/ml), and higher concentrations (30 µg/ml), were found to be 100.57 (0.658), 99.87 (0.358) and 101.55 (0.547), respectively in acetonitrile. Thus, the accuracy of the developed method of docetaxel in acetonitrile was found between 99.87 to 101.55%.

Precision

Precision was determined by studying the repeatability and intermediate precision. In precision study, % RSD values were not more than 2.0% in all the cases. RSD values found for the analytical methods were well within the acceptable range indicating that these methods have excellent precision. (11)

The amount of docetaxel present in the formulation was calculated by using the equation generated by the linearity studies.

LOD and LOQ

The LOD and LOQ of docetaxel were determined using calibration standards. LOD and LOQ were calculated as 3.3 σ/S and 10 σ/S, respectively, where S is the slope of the calibration curve and σ is the standard deviation of y intercept of regression equation.(11,13)

3.4 Conclusion:

The UV-visible spectrum obtained by scanning the 10µg/ml of docetaxel recorded between 200 nm to 400 nm. It was observed that docetaxel shows the characteristic peak at 229 nm and thus it was selected as the analytical wavelength. Analytical method for docetaxel was developed and validated by UV spectrophotometrically in acetonitrile. The high correlation coefficient in the above solvent indicated that absorbance and concentration of the drug was linearly related. Beer's law was found to be obeyed in the range of 5 to 45 µg/ml for docetaxel in acetonitrile.

As a part of method validation, there is negligible difference in the absorbance values of the fresh and the stored solutions indicating that docetaxel is stable over the period of analysis. The accuracy of the developed method of docetaxel in acetonitrile was found to close to 100.00 %, between 98.00 to 102.00 %. It revealed that any small change in the drug concentration in the solutions could be accurately determined by the proposed analytical methods.(7) The results suggest that the methods were very accurate. In precision study, % RSD values were not more than 2.0 % in all the cases. RSD values found for the analytical methods were well within the acceptable range indicating that these methods have excellent precision. The LoD and LoQ for the assay of Docetaxel using Acetonitrile is 0.175 µg/ml and 0.195 µg/ml respectively indicating that the method is sensitive. The developed spectrophotometric methods for determination of Docetaxel are simple, specific, accurate, precise, rapid and economical which indicates its adequacy for routine pharmaceutical analysis. It is concluded that the developed spectrophotometric method can be successfully utilized for the routine estimation of Docetaxel.

Reference List

1. Du P, Li N, Wang H, Yang S, Song Y, Han X, et al. Development and validation of a rapid and sensitive UPLC-MS/MS method for determination of total docetaxel from a lipid microsphere formulation in human plasma. J Chromatogr B Analyt Technol Biomed Life Sci [Internet]. 2013 May 1 [cited 2016 Feb 21];926:101–7. Available from: http://www.sciencedirect.com/science/article/pii/S1570023213000913
2. Andersen A, Warren DJ, Brunsvig PF, Aamdal S, Kristensen GB, Olsen H. High sensitivity assays for docetaxel and paclitaxel in plasma using solid-phase extraction and high-performance liquid chromatography with UV detection. BMC Clin Pharmacol. 2006;6:2.
3. Bissery MC, Gu??nard D, Gu??ritte-Voegelein F, Lavelle F. Experimental antitumor activity of taxotere (RP 56976, NSC 628503), a taxol analogue. Cancer Res. 1991;51(18):4845–52.
4. Cortes JE, Pazdur R. Docetaxel. J Clin Oncol [Internet]. 1995;13(10):2643–55. Available from: http://www.ncbi.nlm.nih.gov/pubmed/7595719
5. Ciccolini J, Catalin J, Blachon M., Durand A. Rapid high-performance liquid chromatographic determination of docetaxel (Taxotere) in plasma using liquid–liquid extraction. J Chromatogr B Biomed Sci Appl [Internet]. 2001 Aug [cited 2016 Feb 21];759(2):299–306. Available from: http://www.sciencedirect.com/science/article/pii/S0378434701002389
6. Engels FK, Mathot R a a, Verweij J. Alternative drug formulations of docetaxel: a review. Anticancer Drugs [Internet]. 2007;18(2):95–103. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17159596\nfile:///Users/andrewmikhail/Documents/Mendeley Desktop/Engels, Mathot, Verweij/2007/Anti-cancer drugs/Alternative drug formulations of docetaxel a review/Engels, Mathot, Verweij - 2007 - Alternative drug formul
7. Malleswara Reddy A, Banda N, Govind Dagdu S, Rao DV, Kocherlakota CS, Krishnamurthy V. Evaluation of the pharmaceutical quality of docetaxel injection using new stability indicating chromatographic methods for assay and impurities. Sci Pharm. 2010;78(2):215–31.
8. Rao BM, Chakraborty A, Srinivasu MK, Devi ML, Kumar PR, Chandrasekhar KB, et al. A stability-indicating HPLC assay method for docetaxel. J Pharm Biomed Anal. 2006;41(2):676–81.

9. Venishetty VK, Parikh N, Sistla R, Ahmed FJ, Diwan PV. Application of validated RP-HPLC method for simultaneous determination of docetaxel and ketoconazole in solid lipid nanoparticles. J Chromatogr Sci. 2011;49(2):136–41.
10. 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;24(7):2019–33.
11. Fda U. Guidance for Industry. Process Validation: General Principles and Practices. US Dep Heal Hum Serv … [Internet]. 2011;(January):22. Available from: http://scholar.google.com/scholar?hl=en&btnG=Search&q=intitle:Guidance+for+Industry+Process+Validation+:+General+Principles+and+Practices#0
12. Aleem H, Zhao Y, Lord S, McCarthy T, Sharratt P. Pharmaceutical process validation: an overview [Internet]. Proceedings of the Institution of Mechanical Engineers, Part E: Journal of Process Mechanical Engineering. 2003. 141-151 p. Available from: www.dekker.com\nhttp://pie.sagepub.com/lookup/doi/10.1243/095440803766612801
13. Huber L. Validation of Analytical Methods [Internet]. Agilent Technologies. 2010. p. 65. Available from: http://www.chem.agilent.com/Library/primers/Public/5990-5140EN.pdf