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DEVELOPMENT AND VALIDATION OF NEW ANALYTICAL METHOD FOR PACLITAXEL IN BULK AND PHARMACEUTICAL DOSAGE FORM BY REVERSE PHASE HPLC (RP-HPLC)

SREERAMAMURTHY PYLA[*1]., K.SRINIVAS[2]., JOGENDRAKUMAR YVV[3]. AND JAGADEESH PANDA[4].

*[*134] Raghu College of Pharmacy, Dakamarri (V), Bheemili (M), Visakhapatnam Dist. A.P, India.*
[2] Sri Vasavi institute of pharmaceutical sciences, T.P.Gudem, E.G District.

ABSTRACT

A simple, precise, rapid and accurate reverse phase HPLC method has been developed for the determination of Paclitaxel in bulk and its pharmaceutical dosage form. An enable C18G, 250mmX4.6mm i.d, 5µm particle size column was used with photo diode array UV-Visible detector. The mobile phase consisting of Phosphate Buffer p^H 5.0 and Acetonitrile in the ratio of 90:10V/V was used. The flow rate was 1ml/min and the effluent was monitored at 282nm. The retention time of the drug was 3.610 minutes. The method was linear over the concentration range of 25-125µg/ml. the method precision for the determination of assay was below 2% RSD. The percentage recovery of paclitaxel was 99.41 – 99.83%. The validation of method was carried out utilizing ICH guidelines.

KEYWORDS: **Paclitaxel, RP- HPLC, Development, Validation.**

SREERAMAMURTHY PYLA
Raghu College of Pharmacy, Dakamarri (V), Bheemili (M), Visakhapatnam Dist. A.P, India.

INTRODUCTION

The main aim of the present study was to develop a simple, precise and accurate reversed phase HPLC method for the estimation of Paclitaxel in bulk drug samples and in pharmaceutical dosage form. Paclitaxel is a mitotic inhibitor used in cancer chemotherapy. It was discovered in a US National Cancer Institute program at the Research Triangle Institute in 1967 when Monroe E. Wall and Mansukh C. Wani isolated it from the bark of the Pacific yew tree, *Taxus brevifolia* and named it taxol. Later it was discovered that endophytic fungi in the bark synthesize paclitaxel. Paclitaxel is used to treat patients with lung, ovarian, breast, head and neck cancer, and advanced forms of Kaposi's sarcoma. Paclitaxel is one of several cytoskeletal drugs that target tubulin. Paclitaxel-treated cells have defects in mitotic spindle assembly, chromosome segregation, and cell division. The ability of paclitaxel to inhibit spindle function is generally attributed to its suppression of microtubule dynamics, but recent studies have demonstrated that suppression of dynamics occurs at concentrations lower than those needed to block mitosis. At the higher therapeutic concentrations, paclitaxel appears to suppress microtubule detachment from centrosomes, a process normally activated during mitosis. The chemical name of Paclitaxel is (2α, 4α, 5β, 7β, 10β, 13α)-4, 10-bis (acetyloxy)-13-{[(2*R*, 3S) - 3-(benzoylamino)-2-hydroxy-3-phenylpropanoyl] oxy} - 1, 7-dihydroxy-9-oxo-5, 20-epoxytax-11-en-2-yl benzoate.

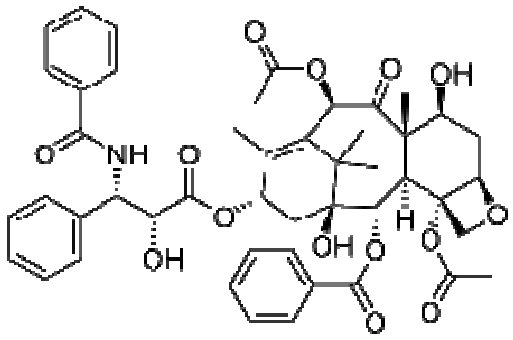

Figure 1.0
Paclitaxel Structure

MATERIALS & METHODS

Chromatographic conditions

Quantitative HPLC was performed on a binary Shimadzu prominence HPLC in Gradient mode with a 20μl sample injection loop (manual), and SPD 20A Photo diode array UV-Visible detector. The output signal was monitored and integrated using LC solutions software. An enable C18G (250 x 4.6 mm, packed with 5 μm) column was used for the separation. To develop a suitable and robust HPLC method for the determination of Paclitaxel different mobile phases methanol: water, Acetonitrile: water, Acetoniotrile: buffer, methanol: buffer were used in different compositions of mobile phases (80:20, 40:60, 55:45, 90:10) at different flow rates (0.5,1.0, 1.2, 1.5, 1.8 ml/min). Then the composition of the mobile phase Phosphate Buffer pH 5.0 and acetonitrile in the ratio of 90:10 at flow rate of 1 ml/ min gave sharp peaks with minimum tailing and good resolution for Paclitaxel. Whereas with other compositions of mobile phases at other flow rates broad peaks

and pronounced tailing was observed. Then, Paclitaxel was eluted at retention times around 3.610 min with symmetric peak shape. Optimized chromatographic conditions were shown in Table1.0

Preparation of Phosphate buffer p^H 5.0

Dissolved 6.8g of Potassium Di Hydrogen Phosphate in 1000ml of water and adjusted the p^H to 5.0 with 10M Potassium Hydroxide.

Preparation of standard drug solution

Stock solution of the drug (pure) was prepared by dissolving 100 mg of Paclitaxel in 50 ml of Acetonitrile in 100 ml volumetric flask and the final volume was made up to 100 ml using Acetonitrile. The working standard solutions were prepared by taking suitable aliquots of drug solution from the standard solution of 100 μg/ml and adjusted the volume with acetonitrile.

Preparation of sample solution from pharmaceutical formulation

Accurately pipette out 5.0 ml paclitaxel equivalent to 30mg of Paclitaxel from the contents of the three vials (ALTAXEL® 5ml vial, Alkem cytomed) and transfered into 10ml volumetric flask containing 5ml of Acetonitrile. The mixture was allowed to stand for 0.5 hr with intermittent sonication to ensure complete solubility of the drug, and filtered through a 0.45μm membrane filter, followed by adding Acetonitrile to obtain stock solution of 3mg/ml. The solution was further diluted stepwise with acetonitrile to get concentrations within the linearity range.

Procedure for calibration curve

The contents of the mobile phase were filtered before use through a 0.45μm membrane filter and pumped from the respective solvent reservoirs to the column at a specified flow rate. Prior to injection of the drug solutions, the column was equilibrated for at least 30 min with the mobile phase flowing through the systems. Then, twenty micro liters of each of standard and sample solutions were injected into the HPLC system for six times and the retention time, average peak areas of analyte were recorded. The linearity range was found to be in between 25-125 μg/ml for Paclitaxel. The linearity range was shown in Table 2.0 and a typical chromatogram of Paclitaxel was shown in Fig-2.0.

Analysis of formulation

The amount of drug present in each pharmaceutical formulation was calculated through peak area of drug by using the standard calibration curve (concentration in μg/ml was taken on x-axis and peak area on y-axis).The results were shown in table 6.0.

Method Validation

Linearity

The linear fit of the system was illustrated graphically. Least square regression analysis was carried out for the slope, intercept and correlation coefficient. The results are presented in Table 2.0 and Calibration curve in figure 3.0.

Precision

The precision of each method was ascertained separately from the peak areas obtained by actual determination of eight replicates of a fixed amount of drug. The percent relative standard deviation and percentage range of errors (at 0.05 and 0.01 confidence limits) were calculated for Paclitaxel and presented in the table 3.0.

Accuracy

To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts (80%, 100%, and 120%) of bulk samples of Paclitaxel along with within the linearity range were taken and added to the pre-analyzed formulation of concentration 40 μg/ml.20 micro litres of each solution was injected in to the RP-HPLC system and run the chromatograms. Then percentage recovery values were calculated. The results were shown in Table 4.0.

System suitability parameters

System suitability parameters are usually developed after method development and

validation has been completed. (Or) The USP (2000) defines parameters that can be used to determine system suitability prior to analysis. The system suitability parameters like Theoretical plates (N), Tailing factor (T), LOD (μg/ml) and LOQ (μg/ml) were calculated and compared with the standard values to ascertain whether the proposed RP-HPLC method for the estimation of Paclitaxel in pharmaceutical formulations was validated or not. The results were shown in Table 5.0.

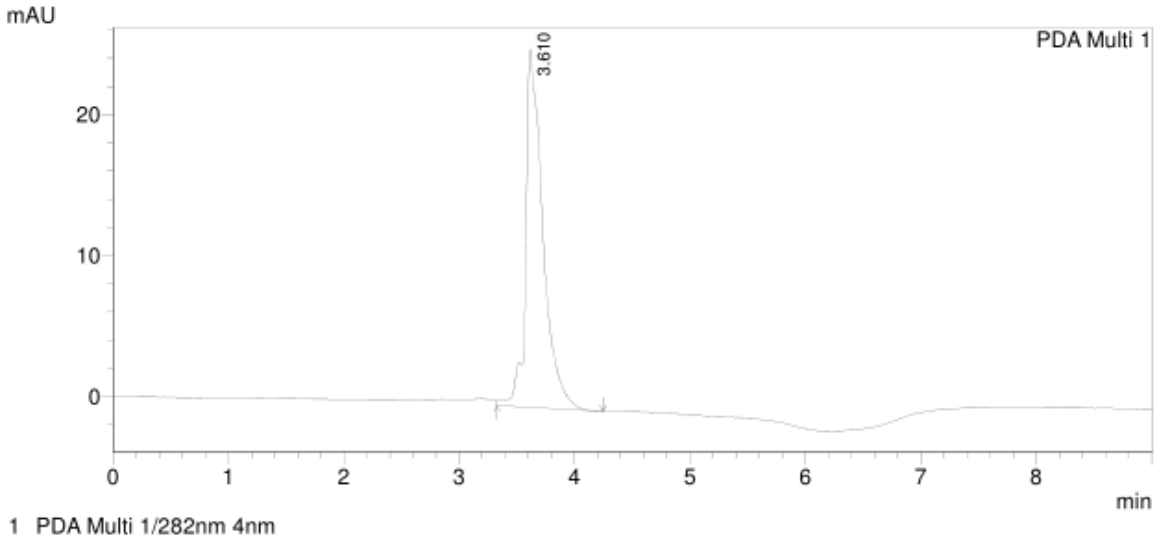

Figure 2.0
Typical chromatogram of Paclitaxel

Table 1.0
Optimized chromatographic conditions

Parameters	Method
Stationary phase (column)	An enable C18G 100 x 4.0mm.
Mobile Phase	Phosphate Buffer pH 5.0: Acetonitrile (90:10)
Flow rate (ml/min)	1.0 ml
Column back Pressure (kgf/cm^2)	165
Run time (minutes)	9
Column temperature (°C)	Ambient
Volume of injection loop (μl)	20
Detection wavelength (nm)	282
Drug RT (min)	3.610

Table 2.0
Linearity

Concentration(μg/ml)	Peak Area	Statistical Analysis
25	64834	Y=2599.1x
50	129986	
75	189682	
100	262855	Correlation coefficient=0.999
125	325689	

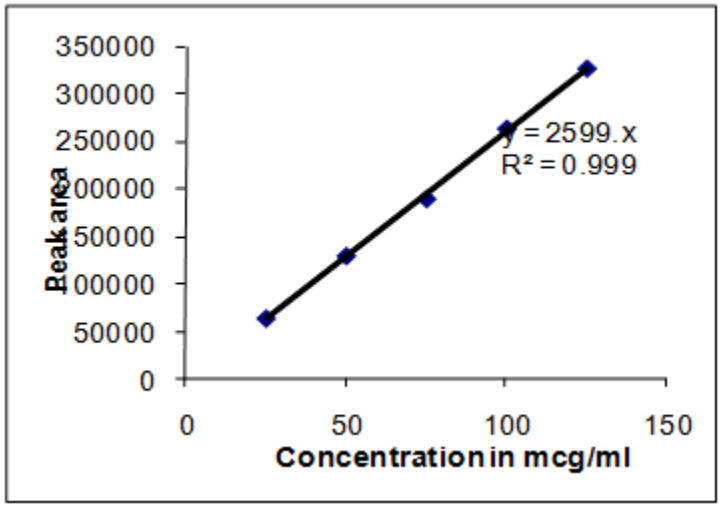

Figure 3.0
Calibration curve of Paclitaxel

Table 3.0
Precision results for Paclitaxel

S.No.	Concentration(µg/ml)	Peak Area	Statistical analysis	
1	50	129864	**Mean**	129405.1
2.	50	129933		
3.	50	128982		
4.	50	128894	**SD**	540.141
5.	50	129853		
6.	50	129981		
7.	50	128879	**% RSD**	0.4
8.	50	128854		

Table 4.0
Accuracy results for Paclitaxel

Sample ID	Concentration (µg/ml)		%Recovery of pure drug	Statistical Analysis	
	Pure drug	Formulation			
S_1 : 80 %	32	40	100.21	Mean	99.79%
S_2 : 80 %	32	40	99.54	SD	0.365
S_3 : 80 %	32	40	99.62	% RSD	0.37
S_4 : 100 %	40	40	100.01	Mean	99.83%
S_5 : 100 %	40	40	99.55	SD	0.25
S_6 : 100 %	40	40	99.95	% RSD	0.25
S_7 : 120 %	48	40	98.99	Mean	99.41%
S_8 : 120 %	48	40	100.26	SD	0. 7361
S_9 : 120 %	48	40	98.98	% RSD	0. 74

Table 5.0
System suitability parameters

S.No	Parameters	Obtained Values
1.	Theoretical plates (N)	21108.916
3.	Tailing factor (T)	1.614
4.	LOD (μg/ml)	0.43
5.	LOQ (μg/ml)	1.413

Table 6.0
Analysis of Paclitaxel present in formulation

S.No	Formulation	Labeled amount (30mg/5 ml)	Amount obtained (mg) proposed Method*	%RSD
1	ALTAXEL	30	29.6224	± 0.560

**** Each value is average of three determinations ± standard deviation.***

RESULTS AND DISCUSSION

From the linearity Table-2.0. It was found that the drug obeys linearity within the concentration range of 25-125 μg/ml for Paclitaxel. From the results shown in precision Table-3.0, it was found that % RSD is less than 2%; which indicates that the proposed method has good reproducibility. From the results shown in accuracy Table-4.0, it was found that the percentage recovery values of pure drug from the pre-analyzed solutions of formulations were in between 99.41 – 99.83%, which indicates that the method was accurate and also reveals that the commonly used excipients and additives present in the pharmaceutical formulations were not interfering the proposed method. The system suitability parameters also reveal that the values were within the specified limits for the proposed method.

CONCLUSION

The proposed method was found to be simple, precise, accurate and rapid for determination of Paclitaxel from pure and its dosage forms. The mobile phase is simple to prepare and economical. Hence, this method can be adopted for routine analysis of Paclitaxel in pure form and its dosage forms and can also be used for dissolution or similar studies.

REFERENCES

1. http://www.rxlist.com/taxol-the internet drug index for paclitaxel drug.
2. Fu Y,LiS,ZuY,Yang G etal.Medicinal chemistry of taxol and its analogues.Curr Med Chem 16:3966-3985(2009)
3. Ciutaru D,Badea I,Lazar I,Tudose A HPLC validated assay of paclitaxels related impurities in pharmaceutical forms containing Cremophor EL.J PharmBiomed Anal 34:493-499(2004)
4. Rajendar G,narayan NGB liquid chromatography –Tandem Mass spectrometry method for Determination of paclitaxel in Human plasma Pharmacetica Analytica Acta 22:189-205(2010)
5. U.S. National Library of Medicine: Drug Information Portal. Drug portal generic name stem showing activity - Paclitaxel.
6. Sung Chul Kim ; YU Jaewon ; Jang won lee ; Park Eun- Seok ; Chi Sang-Cheol; the estimation of paclitaxel in Parenterals by RP-HPLC.Journal pharmaceutical and biomedical analysis , 39, (1-2), 170-176(2005)
7. Shi Shu-yun and Zhou chun-shan; Determination of Paclitaxel in southern yew

tree by HPLC;Journal of Central South University of Technology, 10(3)(2003)

8. Yonemoto Haruo, Nakashima Mihoko, WadaMitsuhiro, Nakashima Ken'ichiro; the estimation of paclitaxel in Parenterals by RP-HPLC; Chromatography; 26(2); 49-50(2005).
9. Mittal Anupama; Chitkara Deepak ; Kumar Neeraj;Spectrophotometric and chromatographic methods for the determination of Paclitaxel in biological fluids; Journal of chromatography. B, 855, 211-219(2007)
10. Florin Marcel Musteata and Janusz Pawliszyn;Determination of free concentrations of Paclitaxel in liposome formulation; J Pharm, Pharmaceut Sci., 9(2):231-237(2006)
11. Lowe, J; Li, H; Downing, KH; Nogales, E (2001). "Refined structure of $alpha;$beta;-tubulin at 3.5 p resolution1". Journal of Molecular Biology 313(2001).