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DEVELOPMENT AND VALIDATION OF NEW RP-HPLC METHOD FOR PACLITAXEL IN BULK AND ITS PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

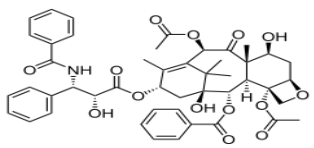
A simple, precise, rapid and accurate reverse phase HPLC method has been developed for the determination of Paclitaxel in bulk and its pharmaceutical dosage form. An enable C18G, 250mm, X4.6mm i.d, 5µm particle size column was used with photo diode array UV-Visible detector. The mobile phase consisting of Acetonitrile and water in the ratio of 80:20V/V was used. The flow rate was 1ml/min and the effluent was monitored at 228nm. The retention time of the drug was 3.737 minutes. The method was linear over the concentration range of 30-250µg/ml. the method precision for the determination of assay was below 2% RSD. The percentage recovery of paclitaxel was 99.41 – 100.83%. The validation of method was carried out utilizing ICH guidelines.

Keywords: Paclitaxel, RP- HPLC, Development, Validation.

INTRODUCTION

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 also used for the prevention of restenosis. Paclitaxel is one of several cytoskeletal drugs that target tubulin. Paclitaxel-treated cells have defects in mitotic spindle assembly, chromosome segregation, and cell division. Unlike other tubulin-targeting drugs such as colchicine that inhibit microtubule assembly, paclitaxel stabilizes themicrotubule polymer and protects it from disassembly. Chromosomes are thus unable to achieve a metaphase spindle configuration. This blocks progression of mitosis and prolonged activation of the mitotic checkpoint triggers apoptosis or reversion to the G-phase of the cell cycle without 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 [1-4].

Paclitaxel



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EXPERIMENTAL

Instrumentation

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 C-18G (250 x 4.6 mm, packed with 5 µm) column was used for the separation [5-7].

Reagents used

a. HPLC water (Qualigens Limited)
b. Acetonitrile (HPLC grade, Finar chemicals Limited)

OPTIMIZATION

Optimization of the method

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, 70:30, 80:20) at different flow rates (0.5,1.0, 1.2, 1.5, 1.8 ml/min). Then the composition of the mobile phase acetonitrile:water in the ratio of 80:20 at flow rate of 1 ml/ min gave sharp peaks with minimum tailing and good resolution for Paclitaxel. Where as 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 3.737 min with symmetric peak shape [8-12]. Optimized chromatographic conditions were shown in Table 1.

METHOD

Preparation of standard drug and solutions

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 [13-15].

Preparation of sample drug solution for pharmaceutical formulations

Accurately pipette out 5.0ml paclitaxel equivalent to 30mg of Paclitaxel from the contents of the three vials (ALTAXEL® 5ml vial, Alkem cytomed) and transfer 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 1.0mg/ml. The solution was further diluted stepwise with acetonitrile and spiked with required amount of standard drug and diluted with mobile phase to get concentrations within the linearity range [16].

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 component were recorded. The linearity range was found to be in between 25-250 µg/ml for Paclitaxel. The linearity range was shown in Table 2 and Calibration curve in figure 2. A typical chromatogram of Paclitaxel was shown in Fig 1.

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) [17].

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.

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. The precision of the assay was also determined in terms of intra-and inter-day variation in the peak areas for a set of drug solutions on three different days. The intra-and inter-day variation in the peak area of the drug solution was calculated in terms of % RSD and the results are presented in the Table 3.

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. From that percentage recovery values were calculated. The results were shown in Table 4.

System suitability parameters

System suitability parameters can be defined as tests to ensure that the method can generate results of acceptable accuracy and precision. The requirements for system

suitability 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), Resolution (R), 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 [18] was validated or not. The results were shown in Table 5.

Table 1. Optimized chromatographic conditions

Parameters	Method
Stationary phase (column)	An enable C-18G 100 x 4.0mm, packed with 5 micron)
Mobile Phase	acetonitrile:water (80:20)
Flow rate (ml/min)	1.0 ml
Column back Pressure (kgf/cm^2)	166
Run time (minutes)	10
Column temperature (°C)	Ambient
Volume of injection loop (µl)	20
Detection wavelength (nm)	228
Drug RT (min)	3.737

Table 2. Linearity

Concentration(µg/ml)	Area	Statistical Analysis
25	751878.7	Y=31801x
50	1563758	
75	2375735	
100	3167515	Correlation coefficient=0.998
150	4751475	
200	6435030	
250	7918787	

Table 3. Precision results for Paclitaxel

S.No.	Concentration(µg/ml)	area	Statistical analysis	
1	50	1563758	**Mean**	1563623
2.	50	1563868		
3.	50	1559989		
4.	50	1564898	**SD**	5540.9
5.	50	1574896		
6.	50	1558989		
7.	50	1556788	**% RSD**	0.35
8.	50	1565798		

Table 4. 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. System suitability parameters

S.No	Parameters	Obtained Values
1.	Theoretical plates (N)	24319
3.	Tailing factor (T)	1.315
4.	LOD (µg/ml)	0.01
5.	LOQ (µg/ml)	0.03

Figure 1. Typical chromatogram of Paclitaxel (Standard drug)

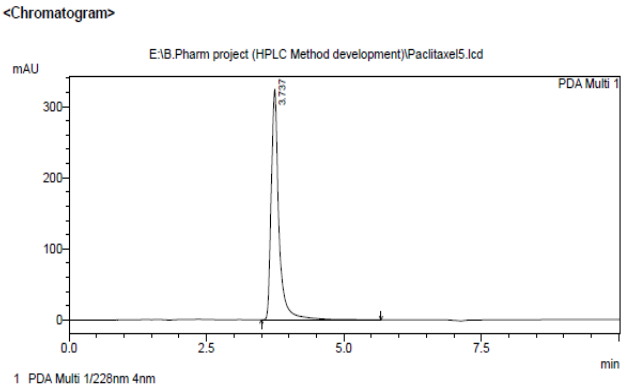
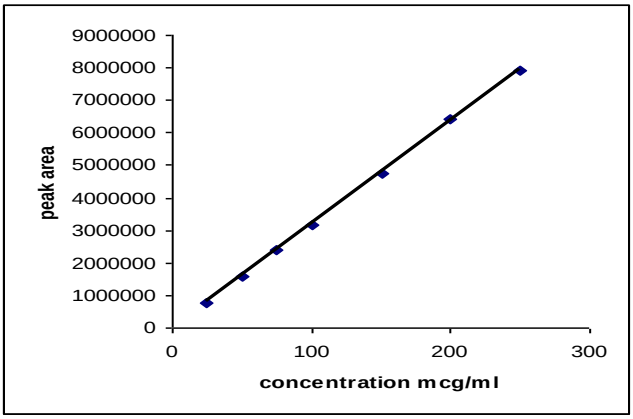


Figure 2. Calibration curve of Paclitaxel



RESULTS AND DISCUSSION

From the linearity Table 2. It was found that the drug obeys linearity within the concentration range of 25-250 µg/ml for Paclitaxel. From the results shown in precision Table 3, 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, 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. The sample recoveries in all formulations were in good agreement with their respective label claims and they suggested non-interference of formulation excipients in the estimation. Hence, this method can be easily and conveniently adopted for routine analysis of Paclitaxel in pure form and its dosage forms and can also be used for dissolution or similar studies.

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