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Validated Spectrophotometric method for estimation of Paclitaxel in Bulk and Pharmaceutical Formulation

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ABSTRACT

A simple, precise, rapid UV method has been developed for the determination of Paclitaxel in bulk and its pharmaceutical dosage form. Paclitaxel is a mitotic inhibitor used in cancer chemotherapy. Paclitaxel is used to treat patients with lung, ovarian, breast, head and neck cancer, and advanced forms of Kaposi's sarcoma. Various methods for analysis of same are available but are time consuming and expensive. Here we have developed new, precise, simple spectrophotometric method for estimation of Paclitaxel from bulk. Medium prepared were selected phosphate buffer PH 10. It showed absorption maxima at 230 nm.This method were validated according to ICH guidelines. The drug obeyed Beers law and showed good correlation. The linearity was observed between 5-55 µg/mL . There was no significant difference in the intraday and interday analysis of Paclitaxel determined. The results of analysis were validated with respect to recovery, linearity; Limit of detection and limit of quantitation were found to be satisfactory.

Keywords: Paclitaxel, UV Visible spectrophotometer, recovery.

INTRODUCTION

The main aim of the present study was to develop a simple, precise, rapid UV 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-{[(2R, 3S) - 3-(benzoylamino)-2-hydroxy-3-phenylpropanoyl] oxy} - 1, 7-dihydroxy-9-oxo-5, 20-epoxytax-11- en-2-yl benzoate.[1]

It is official in British pharmacopoeia[2]. Several analytical techniques have been reported for Paclitaxel with combination i.e. determination including RP High performance liquid chromatography[1], simultaneous estimation by,

HPLC[3] gradient elution by HPLC method[4] and liquid chromatography method for estimation of paclitaxel from parenterals.[5]From this some method are costlier and time consuming.

Objective: The main purpose of this investigation is to develop and validate spectrophotometric method which is simple, rapid, precise and for estimation of Paclitaxel from bulk. This method could also be easily used in routine analytical work and for dissolution studies at very low concentration of Paclitaxel.

MATERIAL AND METHOD

Reagents and Materials: Paclitaxel bulk powder was gifted by Naprod life sciences pvt ltd. Boisar (Thane). The commercial methanol (AR Grade) and anhydrous disodium hydrogen phosphate, Potassium dihydrogen phosphate was procured from Loba Chemicals Ltd, Mumbai, India.

Instrument and Apparatus: A Shimadzu model 1800 (Japan) double beam UV/Visible spectrophotometer with spectral width of 2 nm, wavelength accuracy of 0.5 nm and a pair of 10 mm matched quartz cell was used to measure absorbance of all the solutions.

Methodology and Solvent selection (Solubility studies): These studies were carried out to find a suitable and compatible solvent in which drugs are completely soluble. Paclitaxel readily soluble in methanol and insoluble in Phosphate Buffer PH 10.Therefore methanol is selected as cosolvent and phosphate buffer PH 10used as solvent.

Selection of Analytical Wavelength (λmax): For UV method, analytical wavelength was selected from the spectra of Paclitaxel obtained by using UV spectrophotometer. Stock solution of Paclitaxel was prepared by dissolving 10 mg of drug in 10 ml methanol. From this solution withdraw 0.3 ml and diluted to 10 ml Phosphate buffer PH 10. A concentration of this solution was 30 µg/ml. The working standard solution were scanned in the UV range of 200-400 nm, using Phosphate buffer PH 10 as blank for obtaining the Absorption Maxima (λmax). The maximum absorbance (λmax) for Paclitaxel was found to be 230 nm.

Preparation of Standard Stock Solution: For the preparation of standard 10 mg Paclitaxel was weighed accurately and transferred to a 10 ml volumetric flask and dissolved in methanol. (stock I-1000 µg/ml). From this solution, 0.05 to 5.5 ml of solution was pipetted out and placed into 10 ml volumetric flask. The volume was made up to mark with Phosphate Buffer PH 10 (procedure as per I.P) to give a solution containing 5 to 55 µg/ml.

Method Validation[4,6]:

1. **Linearity:** The stock solution of Paclitaxel1000 µg/ml was prepared and further diluted to obtain solution of range 5-55 µg/ml with Phosphate buffer PH 10. The plot obtained was integrated for various parameters (shown in tab.).

2. **Precision:** The precision studies for the proposed method was carried out as per ICH guideline at the given wavelength (230 nm).The intraday and interday precisions of the proposed methods were determined by estimating the corresponding responses 3 times on the same day and on 3 different days. The data obtained was integrated and found within the specified limits (shown in Tab .).

3. **Limit of Detection and Limit of Quantification:** The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were derived by calculating the signal-to-noise ratio (S/N, i.e., 3.3 for LOD and 10 for LOQ) using the following equations designated by ICH guidelines.

$$LOD = 3.3 \times \sigma/S$$

$$LOQ = 10 \times \sigma/S$$ Where,

σ = the standard deviation of the response and
S = slope of calibration curve.

4. **Accuracy (recovery study):** The accuracy of the methods was determined by calculating the recoveries of Paclitaxel by the standard addition method. Known amounts of standard solutions of Paclitaxel at added at 50, 100 and 150 % level to prequantified sample solutions of Paclitaxel (30µg/ml drug). Amount found as compared to label claim was estimated.

RESULT AND DISCUSSION

Spectral Analysis of Paclitaxel: UV Spectroscopy (Determination of λmax)
Observation: The absorption maximum of the standard solution was scanned between 200-400 nm regions on JASCO UV 550spectrophotometer. The absorption maxima was found to be 230 nm.

U.V.Analysis
Selection of Wavelength: A solution of 30µg/ml solution of drug was prepared in Phosphate Buffer PH 10 and scanned in the range of 200-400 nm, using

the above solvent as blank. The maximum absorbance (λmax) was found to be 230 nm.

Linearity and Range: For the analysis of the linearity the standard stock solution of Paclitaxel 1000 µg/ml was prepared in methanol and further diluted to obtain solution of range 5-55 µg/ml with Phosphate Buffer PH 10. The absorbance was plotted against the corresponding concentrations to obtain the Buffer PH 10. The absorbance was plotted against the corresponding concentrations to obtain the Calibration graph.

Precision:
The precision studies for the proposed method was carried out as per ICH guideline at the given wavelength (230nm).The intraday and interday precisions of the proposed methods were determined by estimating the corresponding responses 3 times on the same day and on 3 different days. The data obtained was integrated and found within the specified limits (shown in Tab 3,4).

Limit of Detection (LOD) & Limit of Quantitation (LOQ)
LOD and LOQ for the method was calculated separately based on the standard deviation response of the calibration curve.

CONCLUSION

From the study it can be concluded that the method described in this paper for the determination of Paclitaxel from bulk and formulation is simple, accurate, sensitive and reproducible. The proposed method utilizes inexpensive solvents. The proposed method could be applied for routine analysis in quality control laboratories.

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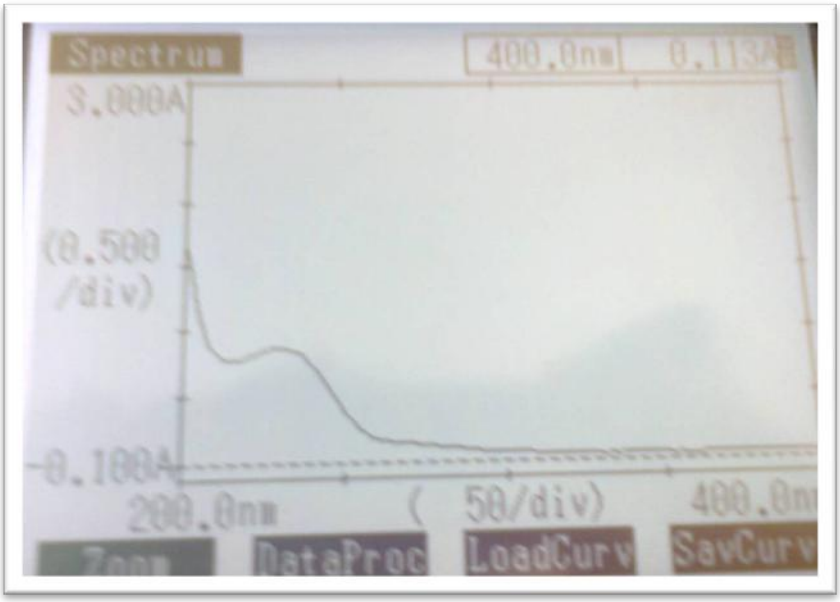

Figure No.1 Scanning of Paclitaxel in Phosphate Buffer pH 10

Table No 1: Observation Table

Conc.	Abs.I	II	III	AVG	SD
0	0	0	0	0	0
5	0.121	0.116	0.118	0.11833	0.002055
10	0.20	0.186	0.192	0.192667	0.005735
15	0.29	0.305	0.296	0.297	0.00755
20	0.367	0.365	0.358	0.363333	0.004726
25	0.433	0.442	0.438	0.437667	0.004509
30	0.544	0.536	0.538	0.539333	0.004163
35	0.619	0.625	0.626	0.623333	0.003786
40	0.709	0.716	0.722	0.715667	0.006506
45	0.778	0.769	0.774	0.773667	0.004509
50	0.869	0.864	0.858	0.866367	0.005508
55	0.969	0.964	0.962	0.965	0.003606

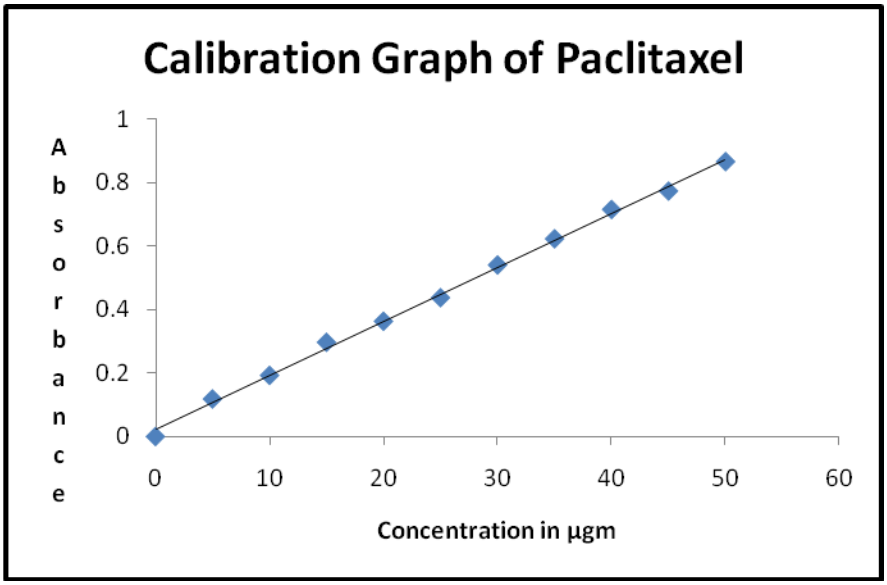

Figure No.2 Calibration Curve of Paclitaxel

Table No 2 –Linear Regression Data for calibration curve.

Parameters.	Results.
λ max (nm)	230nm
Linearity Range, µg/mL	5-55 µg/mL
Slope (m)	0.017056
Intercept (c)	0.02209
Correlation coefficient	0.999216

Table No 3 – Intra-day precision studies.

Parameters	SD*	% RSD
Morning	0.00305	0.5640
Afternoon	0.002	0.3717
Evening	0.00152	0.2938

*Results are average of three determinations.

Table No 4 – Inter-day precision studies.

Parameters	SD*	% RSD
Day First	0.0041	0.1058
Day Second	0.0036	0.6281
Day Third	0.0005	0.1011

**Results are average of three determinations.*

Table No 5 - LOD & LOQ of Paclitaxel

Parameter	µg/mL
LOD(limit of detection)	0.7932
LOQ (limit of quantitation)	2.4038

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