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# Estimation of Paclitaxel drugs by HPLC method

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## Abstract

*Paclitaxel is an anti-leukemic, anti-tumor or in general an anti-neoplastic agent. It was first isolated from the bark of the pacific Yew tree, Taxus breviofolia. Paclitaxel drug is used to treat ovarian, breast and lung cancers. Many pharmaceutical drugs have been analyzed using High Performance Liquid Chromotography method (HPLC). Pharmaceutical manufacturers must maintain tight quality control of their products, and routinely use chromatographic methods for analysis of medications. In the present work, analysis of Paclitaxel drugs such as Nanoxel and Oncotaxel are done by HPLC method. Oncotaxel is widely used chemotherapy agent that is known to have substantial antitumor activity used with a castor oil based solvent, cremophor EL. Whereas, Nanoxel is a polymeric nanoparticle drug delivery agent based on the principle of Nanotechnology and is a cremophor free soluble formulation. HPLC analysis of Paclitaxel drugs was carried out on Shimadzu prominence $C_{18}$ column phenomenex Gemini (250×4.6mm, 5µm) with $KH_2PO_4$ -Acetonitrile (60:40) as the mobile phase and flow rate of 2ml/min at UV detection wavelength of 230nm. The HPLC column separated the basic anti-tumor drug and impurities with good resolution, peak shape and efficiency. The HPLC column was calibrated for peak area at five different concentrations. As the concentration increases the peak area also increases. A plot of mean area versus concentration gave a linear relationship. The linearity coefficient for Nanoxel and Oncotaxel was found to be 0.9997 and 0.9996 respectively. The developed method was found to be reliable and precise for the analysis of Paclitaxel drugs.*

**Key words:** Paclitaxel, HPLC.

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## INTRODUCTION

Paclitaxel is a natural product with antitumor activity. TAXOL (paclitaxel) is obtained via a semi-synthetic process from Taxus baccata. The chemical name for paclitaxel is 5 beta,20-Epoxy-1,2a,4,7 beta,10 beta,13 alpha-hexahydroxytax-11-en-9-one 4,10-diacetate 2-

benzoate 13-ester with (2 R,3S)-N-benzoyl-3-phenylisoserine. Paclitaxel is a white to off-white crystalline powder with the empirical formula $C_{47}H_{51}NO_{14}$ and a molecular weight of 853.9. It is slightly soluble in water, and melts at around 216–217° C. The figure 1 shows the molecular structure of Paclitaxel[1].Literature survey reveals many spectrophotometric and chromatographic methods for the determination of Paclitaxel in biological fluids[2-4]. There are number of analytical methods reported in recent scientific literature for the quantification of Paclitaxel in pharmaceutical dosage form[5-6]. The present work reports the estimation of polymeric nanoparticle Paclitaxel and solvent based Paclitaxel drug which are commercially available. The availability of an HPLC method with high sensitivity and selectivity will be very useful for the determination of Paclitaxel in pharmaceutical formulations. The aim of the study was to develop a simple, precise, and accurate reversed-phase HPLC method for the estimation of Paclitaxel in pharmaceutical dosage form.

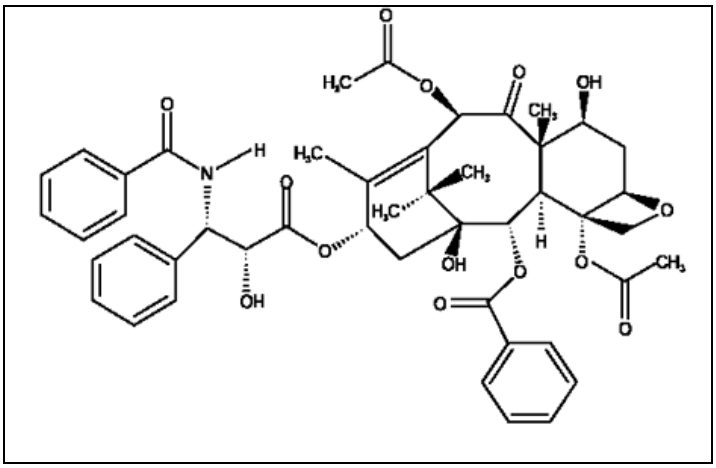

**Fig 1: Molecular structure of Paclitaxel**

## Experimental

Commercially available Paclitaxel injection (Oncotaxel® Vial, Sun Pharmaceuticals Ltd and Nanoxel® vial, Dabur Pharma Ltd) was procured from local market for the present study. The experiment is carried out in Dr.Ceeal laboratory, Chennai.

## Apparatus

An HPLC Shimadzu 1100 series, Shimadzu Technologies, Japan, equipped with an inbuilt solvent degasser, isocratic LC 20 AT, photoiodide array detector with rheodyne injector and an auto sampler, and a reverse phase. ODS $C_{18}$(250x4.6 mm I.d) column.

## HPLC Conditions

The contents of the mobile phase were 0.02 M Potassium dihydrogen phosphate (buffer solution) in water (pH 4.5 adjusted with potassium hydroxide) and acetonitrile in the ratio of 60:40 v/v was used. They were filtered before use through a membrane filter, and pumped from the respective solvent reservoirs to the column at a flow rate of 2ml/min. The run time was set at 8.0 min and the column temperature was ambient. The eluents were monitored at 230 nm.

**Preparation of Sample solution:**
Oncotaxel® (paclitaxel) Injection is a clear, colorless to slightly yellow viscous solution. It is supplied as a nonaqueous solution intended for dilution with a suitable parenteral fluid prior to intravenous infusion. Oncotaxel is available in 30 mg (5 mL), 100 mg (17 mL), and 300 mg (50 mL) multidose vials. Each mL of sterile nonpyrogenic solution contains 6 mg paclitaxel, 527 mg of purified Cremophor® EL (polyoxyethylated castor oil) and 49.7% (v/v) dehydrated alcohol, USP. Accurately pipette out 1ml of Oncotaxel®17ml transferred in to 50 ml volumetric flask containing mobile phase (60:40 v/v buffer and Acetonitrile). The mixture was allowed to stand for few mintues with intermittent sonication to ensure complete solubility of the drug, and then filtered through a membrane filter. An aliquot the 4 ml of this solution was further diluted with 10ml of mobile phase (60:40 v/v buffer:acetonitrile) to give an concentration of Oncotaxel sample solution of 48 mcg/mL

Nanoxel® (paclitaxel) Injection is a polymeric nanoparticle drug. Nanoxel is available in 30 mg (5 mL), 100 mg (17 mL), and 300 mg (50 mL) multidose vials. Each 5mL of sterile nonpyrogenic solution contains 100 mg paclitaxel, and 5ml dehydrated alcohol. It is a Cremophor® EL free solution. Accurately pipette out 0.3ml of Nanoxel® 100mg/5ml vial transferred in to 50 ml volumetric flask containing mobile phase (60:40 v/v buffer and Acetonitrile). The mixture was allowed to stand for few mintues with intermittent sonication to ensure complete solubility of the drug, and then filtered through a membrane filter. An aliquot the 4 ml of this solution was further diluted with 10ml of mobile phase (60:40 v/v buffer:acetonitrile) to give an concentration of Nanoxel sample solution of 48 mcg/mL

**Linearity:**
Aliquots of standard Oncotaxel and Nanoxel 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 Paclitaxel are in the range of 48-72 mcg/ml Each of these drug solutions (20 µL) was injected into the column, and the peak area and retention time were recorded. Evaluation was performed with UV-Visible SPD 20A detector at 230 nm and a calibration graph was obtained by plotting peak area versus concentration. The plot of peak area of each sample against respective concentration was found to be linear in the range of 48-72 mcg/ml. The correlation coefficient of Oncotaxel and Nanoxel was found to be 0.9997 and 0.9996 respectively. Fig 2-3 presents the chromatogram of Oncotaxel and Nanoxel for single concentration.
The linear regression equation being

$$\mathbf{Y = mx + b}$$

*Where,*
*Y= mean peak value, m=slope, b= intercept, x= mean concentration in mcg/ml*

The regression characteristics, such as slope (m), intercept (b) were calculated for this method and given in table 1.

**Table 1: Linear Regression Data for Calibration curves.**

| Drug | Oncotaxel | Nanoxel |
|---|---|---|
| Concentration range (mcg/ml) | 48-72mcg/ml | 48-72mcg/ml |
| Slope(m) | 30.8 | 29.1 |
| Intercept(b) | 70 | 177 |
| Correlation coefficient | 0.9996 | 0.9997 |

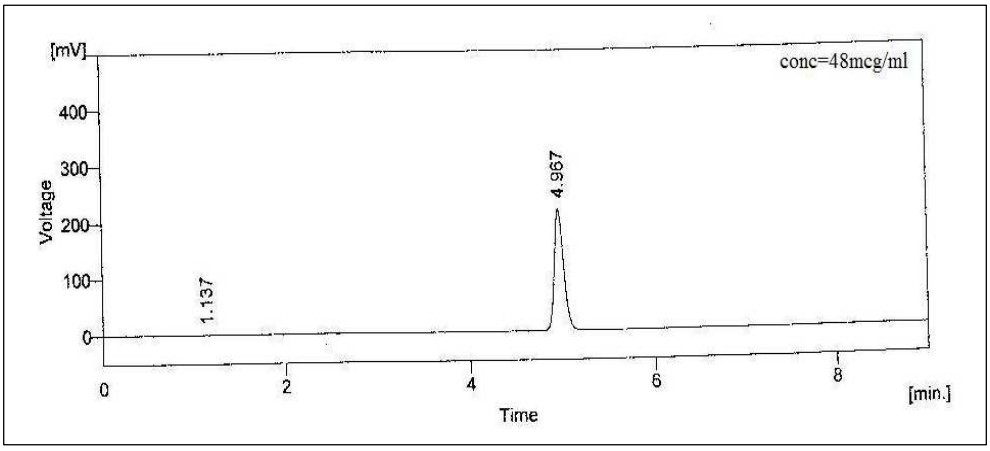


**Fig 2: Chromatogram of Oncotaxel**

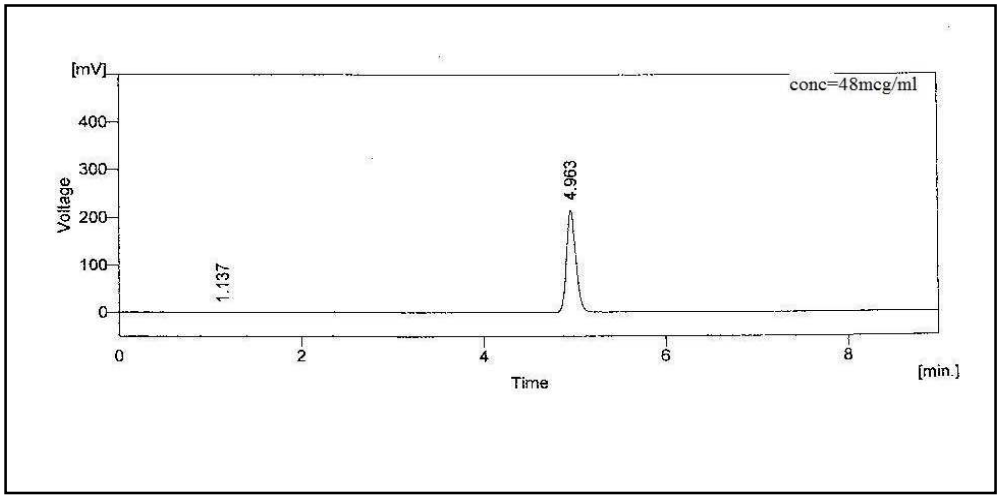


**Fig 3: Chromotogram of Nanoxel**

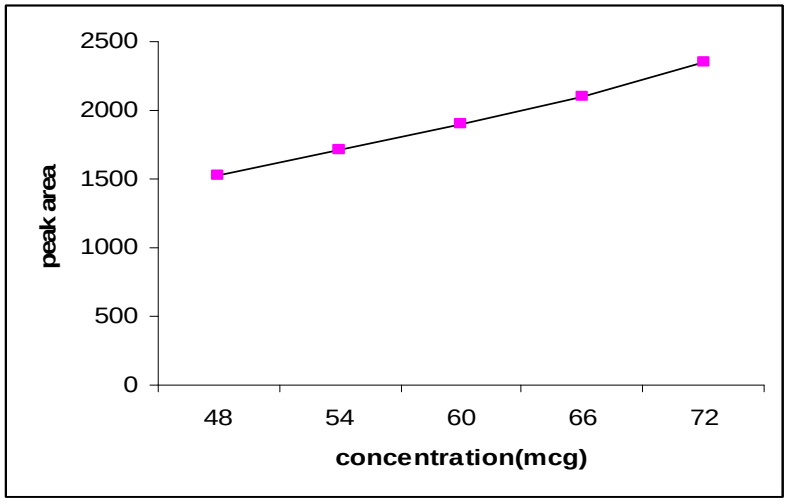


**Fig 4: Linearity curve of Oncotaxel**

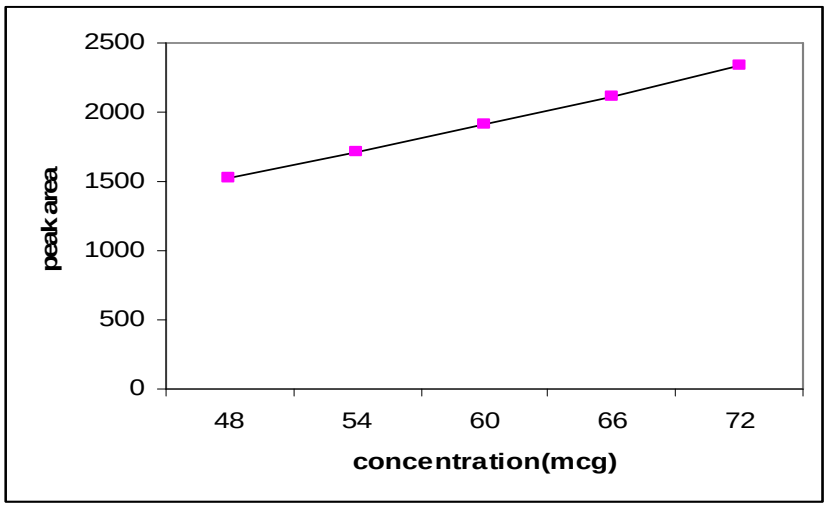


**Fig 5: Linearity curve of Nanoxel**

**Results and Discussions**

The system suitability tests were carried out on prepared sample solutions of Paclitaxel drugs. Parameters that were studied to evaluate the suitability of the system are given in Table 2.

**Limit of Detection (LOD) and Limit of Quantification (LOQ):**

LOD is defined as the quantity or concentration that represents the smallest measure of an analyte that can be detected with reasonable certainty by a given analytical procedure.The limit of detection(LOD) may be expressed as:

$$LOD = 3.3\ \sigma/m$$

*Where,*

*σ=the standard deviation of the response, m= the slope of the calibration curve*

LOQ, is the lowest amount of analyte in a sample that can be quantitatively determined with suitable precision and accuracy. The limit of quatitation (LOQ) may be expressed as:

$$LOQ=10\ \sigma/m$$

The limit of detection (LOD) and limit of quantification (LOQ) for both Paclitaxel drugs were found to be 1.68 and 5.09 mcg/ml respectively for both paclitaxel drugs, since there was no variations in the linearity coefficient for both the drugs under same concentration range. From the typical chromatogram for Oncotaxel and Nanoxel as shown in fig 2-3 for single concentration. It was found that the retention time was 4.967mins for Oncotaxel and 4.963min for Nanoxel. A mixture of acetonitrile and 0.02 M potassium dihydrogen phosphate in water (pH 4.5 adjusted with KOH) in the ratio of 40:60 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 was observed between the concentration range of 48-72 mcg/ml(fig 4-5). The absence of additional peaks in the chromatogram indicates non-interference of the common excipients present in the pharmaceutical dosage form of the Paclitaxel drugs. This demonstrates that the developed HPLC method is simple, linear, accurate, sensitive.

**Table 2: Validation Summary**

| Validation parameter | Results |
|---|---|
| Retention time in minutes<br>Oncotaxel<br>Nanoxel | <br>4.967 min<br>4.963 min |
| LOD | 1.68mcg/ml |
| LOQ | 5.09mcg/ml |

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

## CONCLUSION

The proposed HPLC method is rapid, superior to many reported previously in terms of sensitivity, linear range of response and analysis time. A single chromatographic run took less than 10 min. besides being high sensiive, the procedure is intended to determine Paclitaxel in pharmaceutical formulations, since the method is specific for Paclitaxel under the described condition and is free from the use of internal standard for quantitation. The method can be used to monitor the content uniformity of tablets and injectables, and purity of paclitaxel in raw material.

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