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An approach for validated RP-HPLC method for the analysis of paclitaxel in rat plasma

Nandhakumar Sathyamoorthy, Vijayalakshmi Rajendran, Naveena V.S.H, Magharla Dasaratha Dhanaraju

Dept of Pharmaceutics, GIET School of pharmacy, NH-16 Chaitanya Knowledge city, Rajahmundry-533296, AP, India.

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ABSTRACT

This report describes analysis of paclitaxel, which is an antineoplastic drug used in the treatment of kaposi's sarcoma and cancer of the lung by isocratic high performace liquid chromatography with UV detection in pure form and rat plasma. The analysis was carried out using phenomenex C18 (250 x 4.6 mm, 5 µm particle size) column with a mobile phase consisting of acetonitrile and phosphate buffer (pH 5) in the ratio of 80:20%, v/v. Paclitaxel was eluted at the retention time of 5.3 min when operated at the flow rate of 1 ml/min and monitored by UV at 227 nm. Paclitaxel was extracted from rat plasma by simple LLE method using non- toxic ethyl acetate as extraction solvent. The linearity was accessed in the concentration range of 100-600 µg/mL with correlation coefficient of 0.9999 and percentage recovery of 99.86. The liquid chromatography method was extensively validated for linearity, accuracy, precision, LOD, LOQ and robustness. All these analytical validation parameters were observed to be satisfactory, which indicates the usefulness of method for determination of paclitaxel in pure form and rat plasma. No interfering peaks were observed during the analysis.

INTRODUCTION

Paclitaxel is chemically known as (2α,4α,5β,7β,10β,13α)-4,10-bis(acetyloxy)-13-{ [(2*R*,3*S*)-3-(benzoylamino)- 2- hydroxy- 3- phenylpropanoyl] oxy}-1,7-dihydroxy-9-oxo-5,20-epoxytax-11-en-2-yl benzoate, its empirical formula is $C_{47}H_{51}NO_{14}$ and molecular weight is 853.906. It is slightly soluble in water, and melts at around 216–217°C. The structural complexity of paclitaxel exists in the taxane skeleton with its four-membered oxetane ring and a homochiral ester side-chain at C13. It was demonstrated by extensive investigations that the intact taxane ring with its ester side chain are fundamental for the cytotoxicity of paclitaxel (Fig. 1). The cytotoxic activity if further enhanced by the presence of an accessible hydroxyl group at position 2'' of the ester side-chain (Guenard *et al.*, 1993). Paclitaxel is a novel microtubule agent known to be mediated by binding to tubulin, stabilizing microtubules and blocking the transit of cell cycling from the G_2-phase to the M-phase, and indicated in the treatment of breast cancer after failure of combination chemotherapy. Several assay methods for the determination of paclitaxel by LC (Gouda and Badari.,2010; Kumar *et al.*, 2009; Siddiqui *et al.*, 2012; Jamis-Dow *et al.*, 1993; Mase *et al.*, 1994; Huizing *et al.*, 1995) and capillary electrophoresis (Hempel *et al.*, 1996) and immune assays (Leu *et al.*, 1993; Grothaus *et al.*, 1993) were found during the survey of literature. Most of these methods employ tedious and complicated extraction procedures like SPE or very latest and expensive equipments like LC-MS or tandem LC-MS which are not in the reach of many researchers.

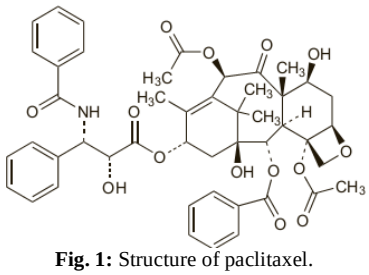

Fig. 1: Structure of paclitaxel.

The present work was aimed to develop a simple and sensitive HPLC method, in economic point of view and useful for regular quantitative analysis of paclitaxel with shorter run time and simple extraction procedure of drug from rat-plasma.

* *Corresponding Author*
Dhanaraju Dasaratha Magharla, Principal and Professor, Department of Pharmaceutics, GIET School of Pharmacy, Rajahmundry; India
mail id: mddhanaraju@yahoo.com

MATERIALS AND METHODS

Equipment

SHIMADZU LC-20AD system with SPD-20A UV-Vis detector equipped with Sphinchrom software; phenomenex C18 column((250 x 4.6 mm, 5 µm particle size) was used for method development, double-beam Perkin Elmer (LAMBDA 25) UV-Vis spectrophotometer was used for spectral measurements and ELICO pH meter was used for pH measurements.

Reagents and standards

Triple distilled water of HPLC grade, acetonitrile of HPLC grade, potassium dihydrogen phosphate (KH_2PO_4) and sodium hydroxide (NaOH), which are of AR grade were used for the experimental work.

Chromatographic conditions

The separation was achieved on RP-column, with a mobile phase of acetonitrile:buffer (pH 5) in the ratio of 80:20, % v/v and at a flow rate of 1 mL/min. The detection was monitored at 227 nm and ambient temperature.

Preparation of mobile phase

Preparation of pH (5) buffer

Approximately 6.8 g of potassium dihydrogen phosphate was dissolved in 1000 ml of water and the pH was adjusted to 5.0 with 10 M sodium hydroxide. Acetronitrile and phosphate buffer were filtered through 0.45 µm membrane filter and sonicated for 20 min before use.

Preparation of stock solution of paclitaxel

About 50 mg of paclitaxel was weighed and transferred into a 50 mL clean dry volumetric flask, 30 mL of diluent was added and sonicated for 5 min and made to the final volume with diluent.

Preparation of plasma samples

Rat blood was mixed with 0.2% EDTA and centrifuged for 10 min at 2000g and the supernatant plasma was collected. To 0.9 mL of plasma, 0.1 mL of stock solution (5 mg/mL) of paclitaxel was added and vortexed for 5 min. About 0.8 mL of ethyl acetate was added, mixed and centrifuged at 3000 g for 10 min and the supernatant organic layer was separated and evaporated under the stream of nitrogen at 40 °C. The residue was diluted as per the linearity range using the diluent and triplicate of 20 µL of plasma sample was injected into the HPLC system at each level.

Calibration curve

Different volumes of stock solutions were accurately transferred to a series of 10 mL volumetric flasks and diluted with acetonitrile to reach 100-600 µg/mL of paclitaxel. Six replicate solutions in the above range were prepared for each concentration. The calibration curve was constructed by plotting the analyte peak area against concentration.

RESULTS AND DISCUSSIONS

Method optimization

The suitable parameters were chosen after several trails with buffers of different pH of 6.8, 6, 5 and 4 with various compositions of methanol, acetronitrile and buffer, however, composition of acetonitrile and buffer of pH 5 was found to be satisfactory. The detection was performed at 227 nm based on the molar absorptivity of the drug, and the UV absorption spectrum was depicted in figure 2. The trails revealed that with the decrease in acetronitrile concentration, the peaks obtained were broad and showed severe tailing. The peak obtained with a composition of acetronitrile and buffer 80:20%, v/v was proved to be most suitable of all the combinations since the peaks obtained were free from tailing. To determine the effect of flow rate, the method was performed at different flow rates 0.5 ml/min, 0.7 mL/min, 0.9 mL/min, 1 mL/min and 1.1 mL/min. The optimum flow rate of 1 mL/min was found best with the retention time of 5.3 min. The chromatogram was shown in figure 3. For LLE from rat plasma several solvents were tried like chloroform, hexane, benzene, diethyl ether, dimethyl formamide, dimethyl sulfoxide and ethyl acetate. Except ethyl acetate other solvents were not found suitable for extraction of paclitaxel as the drug was partially or completely insoluble in them. Hence, ethyl acetate was chosen as organic solvent for liquid-liquid extraction of the drug from rat plasma and the extraction efficiency was found nearer to 95%. LLE method was simplest and cost effective hence the same was adopted for extraction and analysis of the drug.

Method validation

After development of HPLC method for the analysis of paclitaxel, validation of the method was carried out with respect to several parameters like precision, linearity, robustness, ruggedness, accuracy and recovery to ensure that the developed method deals with all the requirements for the aimed intention. A series of solutions were prepared using paclitaxel stock solution and the linearity responses were evaluated across a concentration range of 100-600 µg/ml, pictured in figure. 4 and the linear regression data was provided in table 1.

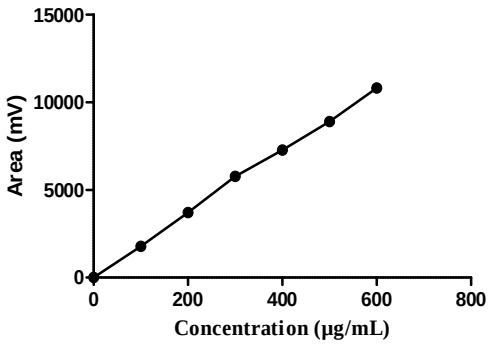

Fig. 4: Linearity plot of paclitaxel by the proposed method.

Table 1: Results showing linearity values of Paclitaxel.

Concentration (µg/mL)	Area (mV)
100	1781
200	3708
300	5771
400	7274
500	8889
600	10810

Table 2: Results showing system precision values of Paclitaxel.

Parameter	Conc (µg/mL)	Mean peak area	SD	% RSD
Inter day	300	5778	15.63	0.17
Intra day	300	5809	16.08	0.27

SD, Standard Deviation; RSD, Relative Standard Deviation

Intraday precision was determined on the same day by repeating the analysis for eight times by observing the peak area of 300 µg/mL of paclitaxel, and on 3 different days for inter day precision. Reproducibility was determined by carrying on the above analytical procedures in different laboratories and the obtained results were compared. Table 3 shows the results for precision studies, indicating good precision of the propose method, (RSD < 1%).

Studies on the effect of robustness on retention time, summarised in table 4, indicates that the results were within the limits of established values thus proving reliability during regular use.

Table 3: Results showing the effect of variation of flow rate.

Flow rate (mL/min)	Rt (min)±SD
0.9	5.86±0.12
1.0	5.33±0.13
1.1	5.28±0.09

SD, Standard deviation

Table 4: Results showing the ruggedness of the method.

Analysts	Rt (min)±SD
Analyst I	5.33±0.9
Analyst II	5.35±0.7

Table 5: Validation summary.

Validation parameters	Results
Theoretical plates(N)	12780
Linearity range, µg/ml	100-600
Tailing factor	0.97
R_t (min)	5.3
LOD, µg/ml	30.7
LOQ, µg/ml	93.1

The results of the ruggedness studies performed by the proposed method were posed in table 5. The LOD and LOQ of the developed methods were determined by analyzing progressively lower concentrations of the standard solutions using optimized chromatographic conditions. The minimum concentration of the standard solution, which gave signal to noise ratio of 3 and 10 were taken as the LOD and LOQ values respectively, which are reported in table 1. Validation was carried out and summary was tabulated in table 5.

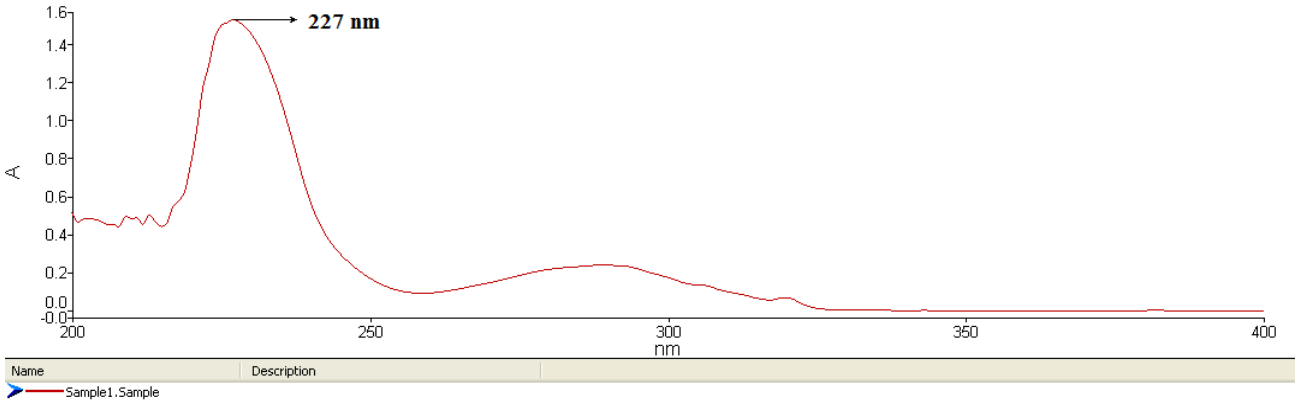

Fig. 2: Absorbance spectra of paclitaxel.

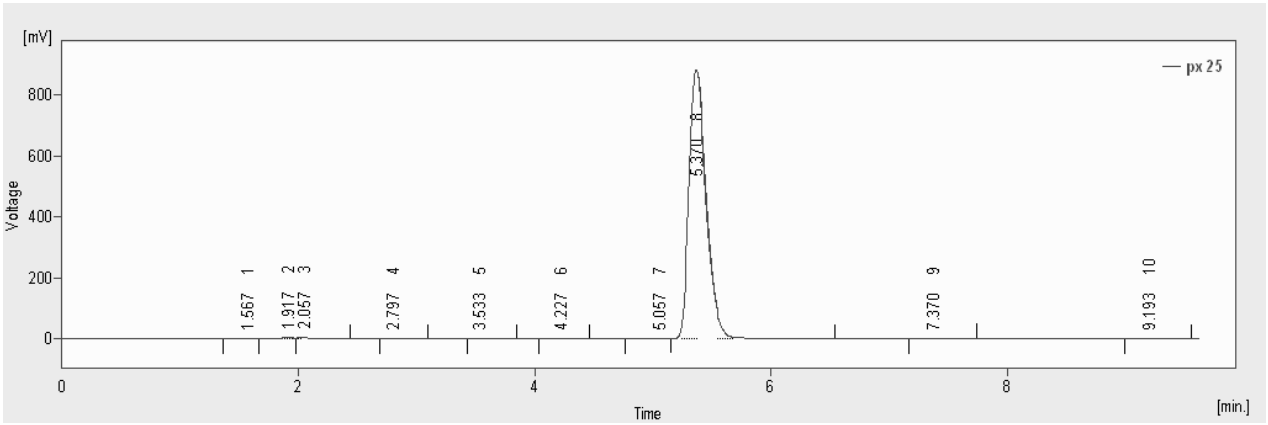

Fig. 3: Chromatogram of paclitaxel in rat plasma.

To assess the accuracy of the aimed method, recovery studies were carried out by standard addition method by spike level to get the concentration of paclitaxel equivalent to 80%, 100% and 120% of the known amount of the drug used for analysis. The average % recovery was calculated and presented in table 6.

Table 6: Results showing accuracy values of paclitaxel.

Sample	%Recovery	% RSD*
80% Sample	99.91	0.7
100%Sample	100.05	0.9
120%Sample	100.05	0.6

*Relative Standard Deviation

CONCLUSION

The results indicate that the proposed RP-HPLC method for the analysis of paclitaxel was precise, accurate, specific and simple. The method was developed and validated according to the ICH guidelines. The results of recovery studies were in good sagreement with the spiked quantity. There was no interference from the plasma components and provided excellent extraction recovery. The extraction procedure is simple with low volume of plasma and extraction solvent. The method was less time consuming and can be employed for routine analysis in laboratories.

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