INTERNATIONAL JOURNAL OF PHARMACY AND ANALYTICAL RESEARCH

ISSN: 2320-2831

IJPAR |Vol.10 | Issue 2 | Apr - Jun -2021

Journal Home page: www.ijpar.com

Research Study Open Access

Analytical method development and validation for the estimation of paclitaxel by using UPLC method

Dr. R. Vani*, Fahmeena Siddiqua

Department of Pharmaceutical Analysis, Shadan Women's College of Pharmacy, Khairatabad, Hyderabad, India

***Corresponding author: Dr. R. Vani**
Email: vrathipelli@gmail.com

ABSTRACT

A simple and precise method was developed for estimating Paclitaxel. The method was found to be specific and precise. The separation was attained on Acquity CSH C18 Column (100*2.0mm &1.5mm) and linearity was achieved in the concentration rage of 25µg/ml to 125µg/ml of Paclitaxel with correlation coefficient 0.99. The percent recovery from the assay was found to be 100.15%for Paclitaxel. Limit of detection and quantitation for Paclitaxel were within the acceptable range. From the stability studies, the percentage variation was less than 10.0% which is the desired criteria. Therefore, this method can be adopted to estimate Paclitaxelin other pharmaceutical formulations.

Keywords: Paclitaxel, UPLC, Method development, Linearity, Validation.

INTRODUCTION

Paclitaxel is a chemotherapeutic agent marketed under the brand name Taxol among others. Used as a treatment for various cancers, paclitaxel is a mitotic inhibitor that was first isolated in 1971 from the bark of the Pacific yew tree which contains entophytic fungi that synthesize paclitaxel. It is available as an intravenous solution for injection and the newer formulation contains albumin-bound paclitaxel marketed under the brand name Abraxane.[1-3] Used in the treatment of Kaposi's sarcoma and cancer of the lung, ovarian, and breast. Abraxane® is specifically indicated for the treatment of metastatic breast cancer and locally advanced or metastatic non-small cell lung cancer. Paclitaxel interferes with the normal function of microtubule growth. Whereas drugs like colchicines cause the depolymerization of microtubules in vivo, paclitaxel arrests their function by having the opposite effect; it hyper-stabilizes their structure. This destroys the cell's ability to use its cytoskeleton in a flexible manner. Specifically, paclitaxel binds to the β subunit of tubulin. Tubulin is the "building block" of microtubules, and the binding of paclitaxel locks these building blocks in place. The resulting microtubule/paclitaxel complex does not have the ability to disassemble. This adversely affects cell function because the shortening and lengthening of microtubules (termed dynamic instability) is necessary for their function as a transportation highway for the cell. Chromosomes, for example, rely upon this property of microtubules during mitosis.[4-8] Further research has indicated that paclitaxel induces programmed cell death (apoptosis) in cancer cells by binding to an apoptosis stopping protein called Bcl-2 (B-cell leukemia 2) and thus arresting its function.

From the literature survey, it was revealed that few UV spectrophotometric methods were developed but were not economical. Moreover, RP-HPLC[9-11] and LC-MS and derivative methods were also developed which estimates Paclitaxel. In the present research work, a new method was developed to estimate Paclitaxel and validated as per ICH guidelines.[12] (Figure 1)

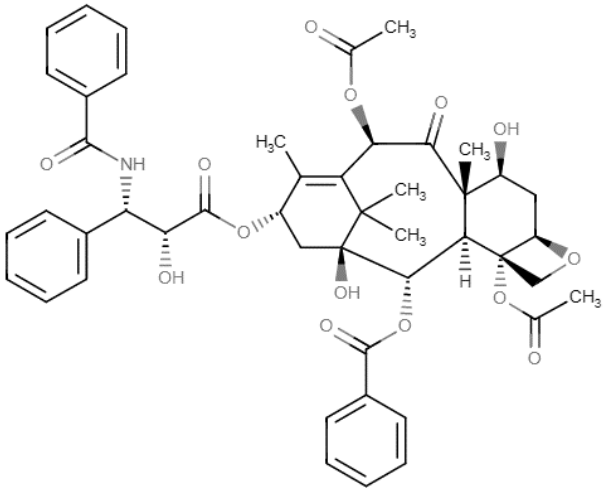

Figure 1: Structure of Paclitaxel

MATERIALS AND METHODS

Gift samples of Paclitaxel were received from Celon Pharma. KH2PO4 was purchased from Final chemicals where as water, aceto nitrile, methanol for HPLC and ortho phosphoric acid was purchased from Merck.

Instrumentation: Agilent Infinity 1290, Hamilton Syringe was used for the estimation of Paclitaxel. UV/VIS spectrophotometer (Thermo Electron Corporation) was used for detection. Instruments such as; pH meter used was of Adwa — AD 10100 and weighing machine was of Afcoset ER-1000A.

Method development

Preparation of Standard solution: 10 mg of Paclitaxel was weighed and exchanged in to 100 ml volumetric jar and broken up in portable stage and after that make up to the check with portable stage and plan 10 µg /ml of arrangement by weakening 1ml to 10ml with portable stage.

Preparation of Sample solution: Weigh amount of powder proportionate to 100mg of Paclitaxel and exchanged in to 100 ml volumetric carafe and broken up in versatile stage and after that make up to the check with portable stage and plan 10 µg /ml of arrangement by weakening 1ml to 10ml with versatile stage.

Procedure: Mixture of Methanol, Acetonitrile and Water in the ratio of 50:30:20%V/V/V was used as mobile phase which was injected into the system for 30 minutes prior to injecting the prepared solutions of standard as well as sample. Detection of the drug was achieved at the wavelength of 254nm at room temperature. After several trials, method was optimized followed by validation of the method considering various validation parameters.

RESULTS AND DISCUSSION

Method development was achieved using Acquity CSH C18 Column (100*2.0mm &1.5mm). Mobile phase was mixture of Methanol, Acetonitrile and Water (50:30:20% v/v/v). Flow rate (1ml/min) and injection volume (10µl) was set. The peaks obtained had good resolution with the retention time 1.455min. Chromatogram of optimized trial is shown in figure 2.

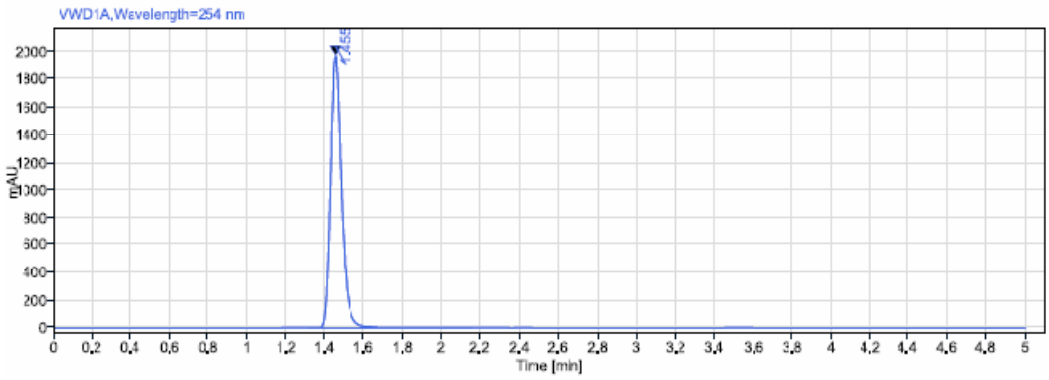

Figure 2: Chromatogram of optimized trial

System Suitability & System Precision: All the parameters were evaluated by performing system suitability studies. The recorded responses for System Suitability & System Precision are depicted in table 1.

Table 1: Results of system suitability parameters

Injection	RT	Peak area	Theoretical plates (TP)	Tailing factor (TF)
1	1.457	7561.87	3579	1.32
2	1.457	7544.48	3619	1.25
3	1.458	7554.34	3591	1.31
4	1.459	7557.49	3628	1.25
5	1.460	7552.46	3631	1.27
6	1.461	7664.35	3630	1.30
Mean	1.459	7572.498	-	-
SD	0.002	45.368	-	-
%RSD	0.112	0.599	-	-

Method validation: Validation of the method was evaluated for various parameters which include linearity, specificity, robustness and stability. The method was also evaluated for specificity of the method and was found to be specific as there were no interactions found. Linearity obtained was shown to have good correlation as shown in table 2.

TABLE 2: Linearity Relationship

S.No	Parameter	PACLITAXEL
1	Correlation coefficient	0.9995
2	Slope	104.17
3	Intercept	314.48

Linearity: The linearity range was observed from 25µg/ml to 125µg/ml of Paclitaxel. The respective absorbance values are depicted in table 3. The linearity graph plotted is presented in figure 3.

Table 3: Linearity results

S.No	Concentration (µg/mL)	Area
1	25	2257.66
2	50	4949.50
3	75	7557.22
4	100	9947.19
5	125	12780.21

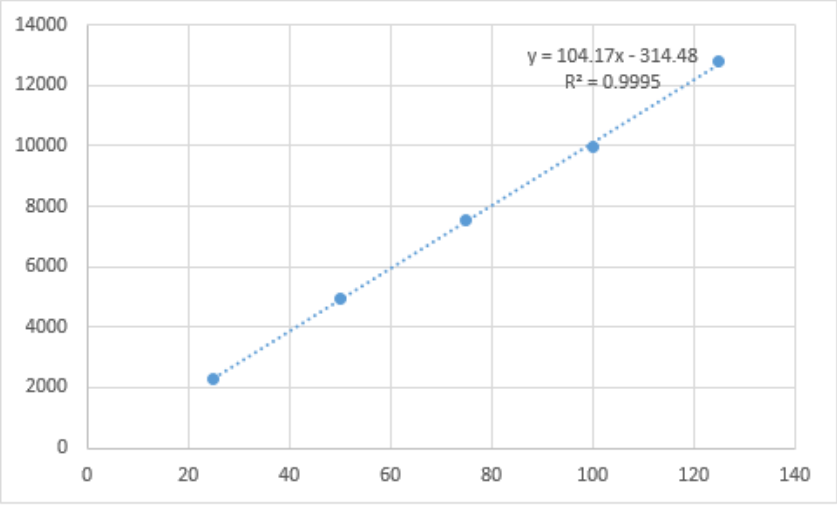

Figure 3: Linearity graph for Paclitaxel

Accuracy: Percent recovery of sample solutions at different concentrations (50%, 100%, and 150%) was calculated. The Percent recovery of Paclitaxelis depicted in table 4.

Table 4: Accuracy (recovery) data for Paclitaxel

Recovery	Area	Avg Area	% Recovered	% Recovery
25	1889.25	1885.78	24.95	99.82
	1878.34			
	1889.74			
75	5667.75	5676.28	75.11	100.15
	5679.65			
	5681.44			
125	9446.25	9451.94	125.08	100.06
	9452.34			
	9457.22			

Limit of Detection and Quantitation: Lowest concentrations of the sample were prepared and measured for LOD and LOQ. The LOD for this method was found to be 0.58µg/ml Paclitaxel. The LOQ for this method was found to be 1.77µg/ml Paclitaxel.

$$LOD = \frac{3.3\sigma}{S}$$
$$= 3.3 * (18.47)/104.17$$
$$= 0.58\mu g/ml$$
$$LOQ = \frac{10\sigma}{S}$$
$$= 10* (18.47)/104.17$$
$$= 1.77\mu g/ml$$

Where

σ = the standard deviation of the response

S = the slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte.

Robustness: The standard and samples were injected by changing the conditions of chromatography. There was no change observed in the parameters like tailing factor, resolution, plate count and asymmetric factor. Chromatograms for variation in flow rate are presented in figure 5 and 6where as chromatograms for variation in temperature are presented in figure 7 and 8. Their respective results are depicted in table 5.

Variation in flow

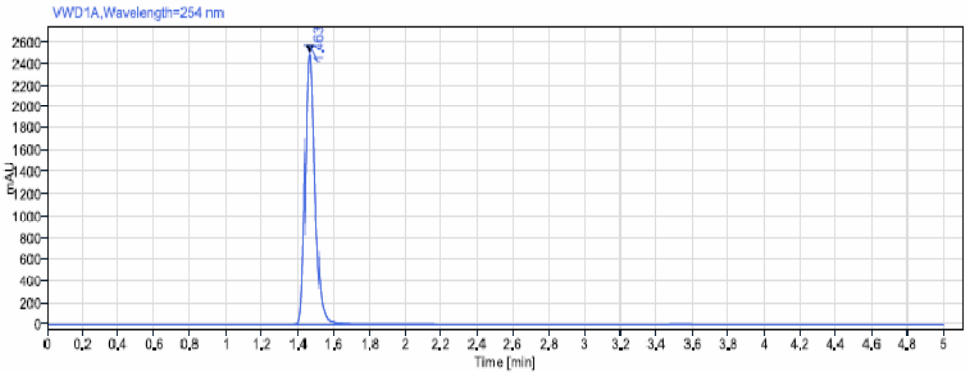

Figure 4: Chromatogram showing less flow

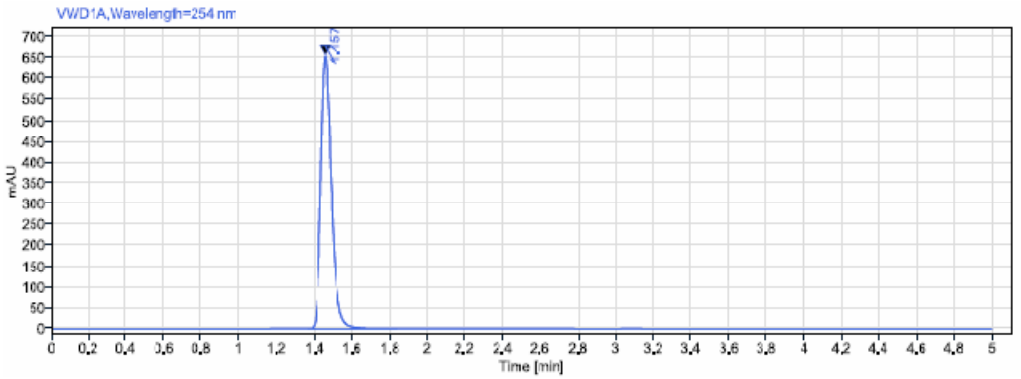

Figure 5: Chromatogram showing more flow

Variation of temperature:

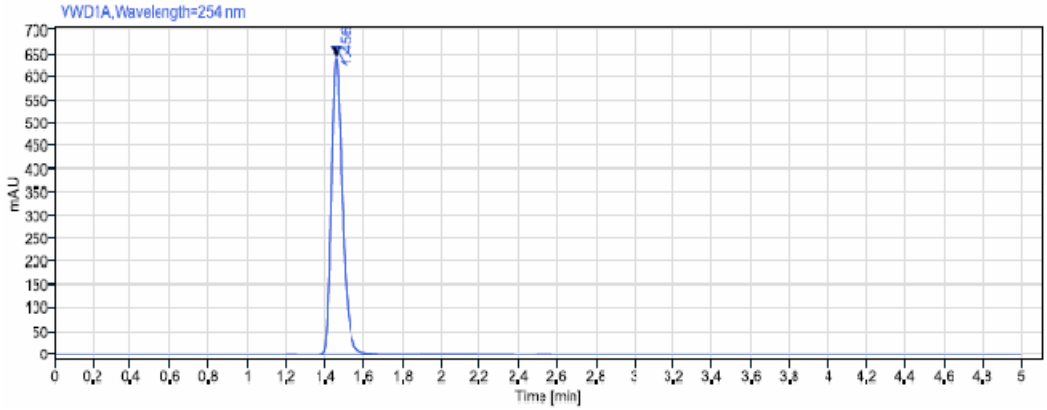

Figure 6: Chromatogram with less temperature

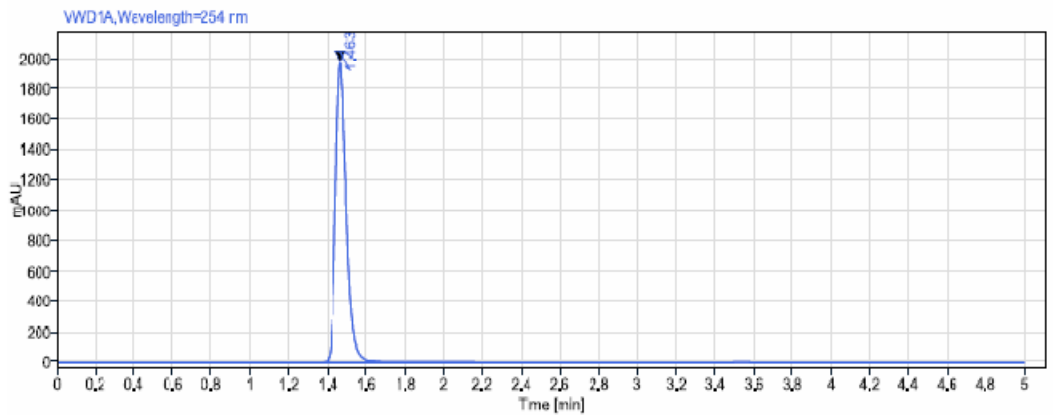

Figure 7: Chromatogram with more temperature

TABLE 5: Results for variation for Paclitaxel

Chromatographic changes		Retention time(min)	Tailing Factor	Theoretical Plates
Flow rate (mL/min)	0.8	1.463	1.32	3672
	1.2	1.457	1.27	3622
Temperature (°C)	25	1.456	1.24	3675
	35	1.463	1.30	3644

CONCLUSION

From the above experimental results and parameters it was concluded that, this newly developed method for the estimation of Paclitaxel was found to be simple, precise, accurate and high resolution and shorter retention time makes this method more acceptable and cost effective and it can be effectively applied for routine analysis in research institutions, quality control department in industries, approved testing laboratories, bio-pharmaceutical and bio-equivalence studies and in clinical pharmacokinetic studies in near future.

REFERENCES

1. Wall ME, Wani MC. Camptothecin and Taxol: discovery to clinic--thirteenth Bruce F. Cain Memorial Award Lecture. Cancer Res. 1995 Feb 15; 55(4):753-60. PMID 7850785.
2. Wani MC, Taylor HL, Wall ME, Coggon P, McPhail AT. Plant antitumor agents. VI. The isolation and structure of Taxol, a novel anti leukemic and antitumor agent from Taxus brevifolia. J Am Chem Soc. 1971 May 5; 93(9):2325-7. doi: 10.1021/ja00738a045, PMID 5553076.
3. Fuchs DA, Johnson RK. Cytologic evidence that Taxol, an antineoplastic agent from Taxus brevifolia, acts as a mitotic spindle poison. Cancer Treat Rep. 1978 Aug; 62(8):1219-22. PMID 688258.
4. Saville MW, Lietzau J, Pluda JM, Feuerstein I, Odom J, Wilson WH, Humphrey RW, Feigal E, Steinberg SM, Broder S, et al. Treatment of HIV-associated Kaposi's sarcoma with paclitaxel. Lancet. 1995 Jul 1; 346(8966):26-8. doi: 10.1016/s0140-6736(95)92654-2, PMID 7603142.
5. Authors. ABI 007. Drugs R D. 2004; 5(3):155-9. doi: 10.2165/00126839-200405030-00003, PMID 15139776.
6. Gaitanis A, Staal S. Liposomal doxorubicin and nab-paclitaxel: nanoparticle cancer chemotherapy in current clinical use. Methods Mol Biol. 2010; 624:385-92. doi: 10.1007/978-1-60761-609-2_26, PMID 20217610.
7. de Bont HBA, Leenders RGG, Scheeren JW, Haisma HJ, de Vos D. Paclitaxel pro drugs, method for preparation as well as their use in selective chemotherapy U.S Patent US5760072, issued September; 1989.
8. Peltier S, Oger JM, Lagarce F, Couet W, Benoît JP. Enhanced oral paclitaxel bioavailability after administration of paclitaxel-loaded lipid nano capsules. Pharm Res. Jun 2006; 23(6):1243-50. doi: 10.1007/s11095-006-0022-2, PMID 16715372.
9. Zhang S-Q, Chen G-H. Determination of paclitaxel in human plasma by UPLC-MS-MS. J Chromatogr Sci. 2008 Mar; 46(3):220-4. doi: 10.1093/chromsci/46.3.220.
10. Jain A, Gulbake A, Jain A, Shilpi S, Hurkat P, Kashaw S, Jain SK. Development and validation of the HPLC method for simultaneous estimation of paclitaxel and topotecan. J Chromatogr Sci. 2014; 52(7, Aug):697-703. doi: 10.1093/chromsci/bmt101, PMID 23843442.
11. Xia XJ, Peng J, Zhang PX, Jin DJ, Liu YL. Validated HPLC method for the determination of paclitaxel-related substances in an intravenous emulsion loaded with a paclitaxel-cholesterol complex. Indian J Pharm Sci. 2013; 75(6):672-9. PMID 24591742.
12. ICH. Validation of analytical procedures: methodology, guidance for industry. Vol. Q2B [guideline]; 1996. p. 1-10.