Pharm Methods, 2017; 8(1): 17-21
A multifaceted peer reviewed journal in the field of Pharm Analysis and Pharmaceutics
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Original Article

Validated RP-UFLC Method Development of Paclitaxel in Pure and Pharmaceutical Dosage Form

Manusmitha Sriram Srinivasa, Ravandur Shivanna Chandan*, Venkata Sairam Koganti

Department of Pharmaceutical Chemistry, JSS College of Pharmacy, JSS University, Sri Shivarathreeshwara Nagar, Mysuru-570 015, Karnataka, INDIA.

ABSTRACT

Introduction: An accurate, precise and rapid method for analysis and quantification of paclitaxel by reverse phase ultra-fast liquid chromatography (RP UFLC) was developed and validated. Paclitaxel in bulk and formulations were analyzed and quantified. **Methods**: Paclitaxel in bulk and formulations were analyzed on phenomenex C18 column (250 mm×4.6 mm i.d., 5 µm particle size) as stationary phase. Mobile phase was composed of acetonitrile and phosphate buffer pH 4.5 in the ratio 50:50 at a flow rate of 1.0 mL/min. Elutes were analyzed using PDA detector at a detection wavelength of 282 nm. The proposed method was validated by ICH harmonized Tripartite guidelines, Validation of Analytical Procedures: Text and Methodology Q2 (R1). **Results**: In this study, the chromatographic peaks of paclitaxel showed good resolution with retention time of 6.5 min. Paclitaxel showed an excellent linearity with 0.994 of correlation coefficient. Other validation parameters including precision, specificity, accuracy, and robustness demonstrated good reliability in the quantification of paclitaxel. **Conclusion:** Thus the newly developed and validated method can be conveniently used for the quantification of paclitaxel in bulk and formulation. The method can also be applied to multicomponent drug analysis.

Key Words: Paclitaxel, RP-UFLC, PDA, Accuracy, Precision.

Correspondence:
R.S. Chandan, Department of Pharmaceutical Chemistry, JSS College of Pharmacy, JSS University, Sri Shivarathreeswara Nagar, Mysuru-570 015, Karnataka, INDIA.
Telephone: +91-821-2548353
Fax Number: +91-821-2548359
Phone no: +91-9900622911

E-mail: rschandan@jssuni.edu.in
DOI : 10.5530/phm.2017.8.3

INTRODUCTION

Paclitaxel is an antineoplastic agent used in the treatment of ovarian cancer, breast cancer and lung carcinomas. It is commonly called as taxol since it is being isolated from the bark of the Pacific yew, *Taxus brevifolia*.[2] Paclitaxel is a cytoskeletal drug that target tubulin. It stabilizes the microtubule polymer and protects it from disassembly and thereby preventing the chromosomes to achieve a metaphase spindle configuration.[5] This blocks the progression of mitosis and prolonged activation of the mitosis triggersapoptosis.

Literature survey reveals several analytical methods have been developed for estimation of paclitaxel in pharmaceutical dosage forms and biological samples including high performance liquid chromatography(HPLC),[1-4] simultaneous estimation of paclitaxel and topotecan by HPLC,[5] stress degradation studies by HPLC,[6] and liquid chromatography-mass spectrometry (LC-MS) methods.[7,8] However, these reported chromatographic methods for estimation of paclitaxel possess multiple drawbacks like sample preparation, low sensitivity, complex mobile phase mixture, strict monitoring of critical method parameters like mobile phase flow rate, column temperature, flow gradient, maintenance of pH, etc. This calls for the development of a simple, rapid, sensitive, efficient and reliable liquid chromatographic method for quantification of paclitaxel in bulk drug and pharmaceutical dosage forms.

Molecular formula and molecular weight of paclitaxel are $C_{47}H_{51}NO_{14}$ and 853.90614. It is insoluble in water.[7] Chemically paclitaxel (Figure 1) is 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.[1]

MATERIALS AND METHODS

Chemicals and reagents

Reference standard of paclitaxel (having assigned purity >99% w/w) was used to develop the new RP-UFLC method. HPLC grade acetonitrile was obtained from Merck specialties Pvt. Ltd. (Mumbai, India). Other chemicals and reagents were analytical grade. Water for RP-UFLC was prepared using Millipore purification system (Direct-Q, Bangalore, India). Intavenous formulation Kansure, Khandewal Laboratories Pvt Ltd, India containing 100 mg/16.7 mL and Intaxel, Fresenius Kabi India Pvt Ltd, containing 6 mg/mL was purchased from the local pharmacy.

Instrumentation

The ultra-fast liquid chromatography (UFLC) used was of Shimadzu Prominence LC-20AD equipped with a 1260 binary pump VL (35MPa), Prominence SIL-20ACHT Auto sampler, and Prominence SPD-M20A Diode array detector. Data collection and analysis were performed using LC solution. Quantification of paclitaxel was achieved using phenomenex C_{18} column. The mobile phase was composed of acetonitrile and phosphate buffer pH 4.5 in the ratio 50:50 v/v. The optimized chromatographic conditions are shown in Table 1.

Preparation of Phosphate buffer pH 4.5

5.04 g of disodium hydrogen phosphate and 3.01 g of potassium dihydrogen phosphate was dissolved in sufficient water to produce 1000 mL. pH was adjusted with glacial acetic acid. Mobile phase was filtered through membrane filter (0.2 µ) prior to use.

Preparation of standard solution of paclitaxel

Standard stock solution of paclitaxel was prepared by transferring 50 mg of drug in to 50 mL of clean volumetric flask having 10 mL of acetonitrile and was ultrasonicated for 5 min. Finally the volume was made up with acetonitrile which gave 1000 µg/mL solution. 100 µg/mL of working standard solution was prepared by taking suitable aliquot from standard stock solution and volume was made up with acetonitrile.

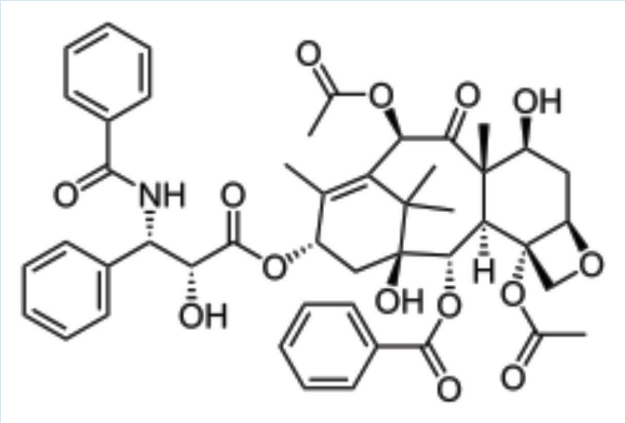

Figure 1: Paclitaxel.

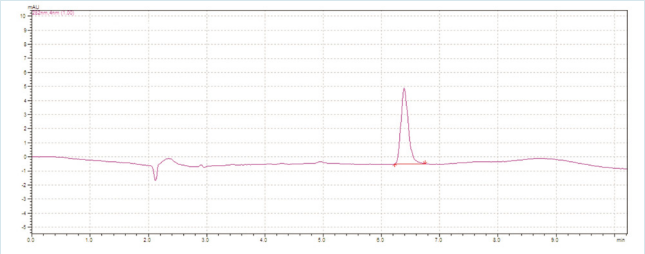

Figure 2: Sample 1 (Intaxel) chromatogram.

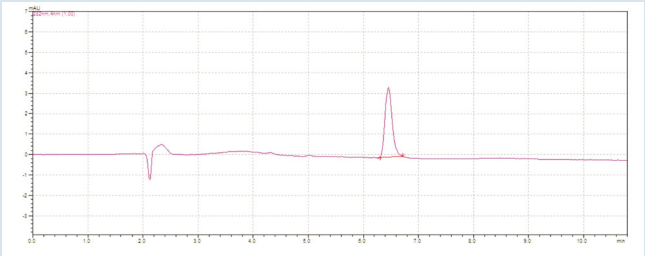

Figure 3: Sample 2 (Kansure) chromatogram.

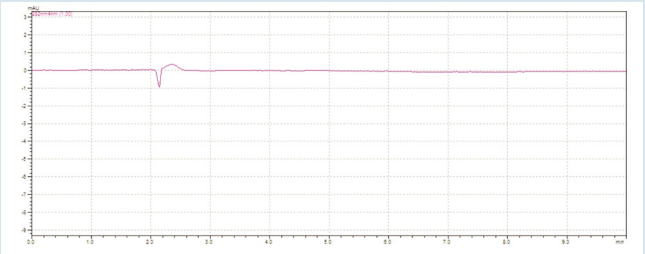

Figure 4: Blank chromatogram.

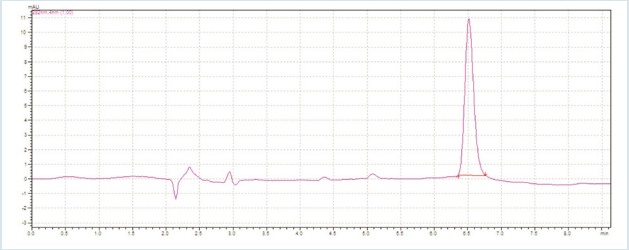

Figure 5: Chromatogram of 10 µg/mL of paclitaxel.

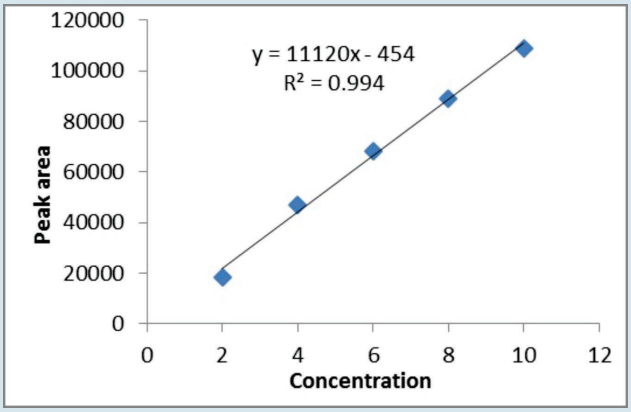

Figure 6: Calibration curve of paclitaxel.

Assay procedure

1mL of the intravenous injection containing 6 mg was transferred into a clean 10 mL volumetric flask. It was dissolved using 5 mL of acetonitrile and ultrasonicated for 5 min. Finally volume was made up to 10 mL using acetonitrile. Solution was filtered by 0.45 µm filter to remove particulate matter, if any. The filtered solution was further diluted for analysis, to get a test concentration of 6 µg/mL. Assay results are tabulated in Table 2 and chromatograms from Figure 2 and 3.

METHOD VALIDATION AND RESULTS AND DISCUSSION

The developed RP-UFLC method was validated as per ICH guidelines.[9]

Linearity and range

Stock solution of paclitaxel (100 µg/mL) was suitably diluted with acetonitrile to get concentrations in the linearity range of 2 to 10 µg/mL. A sample volume of 10 µL was injected onto the column in triplicate, for each solution. Chromatograms, peak area and retention times of each solution were recorded. Calibration curve of paclitaxel was prepared by selecting the concentration (µg/mL) on x-axis and average peak areas on y-axis (Figure 6 and Table 3). The calibration curve data was further subjected to statistical analysis to find out the slope intercept and correlation of coefficient. R^2 for paclitaxel was found to be 0.994 (Table 4). Figure 4 and 5 are the chromatograms of blank and 10 µg/mL of paclitaxel.

Table 1: Chromatographic conditions

Parameters	Methods
Stationary phase	PhenomenexC_{18} column(250×4.6 mm, 5 micron)
Mobile phase	Acetonitirle: Phosphate buffer pH 4.5(50:50)
Flow rate(mL/min)	1.0 mL
Elution	Gradient
Run time (minutes)	10
Column temperature (°C)	Ambient
Volume of injection loop (µL)	10
Detector	PDA
Detection wavelength (nm)	282
Drug RT (min)	6.5

Table 2: Assay of formulation

Brand name	Available form	Label claim	Amount found	Assay
Intaxel	IV Injection	5.882 mg/mL	5.834 mg	99.18%
Kansure	IV Injection	5.988 mg/mL	5.94 mg	99.20%

Table 3: Calibration curve of paclitaxel

Concentration(µg/mL)	Peak area
2	18245
4	46898
6	67311
8	88821
10	108434

Table 4: Linearity data of paclitaxel

Parameters	Paclitaxel
Linearity	2-10 µg/mL
Regression equation	y=11120x-454
Slope	11120
Intercept	454
Correlation coefficient	0.994
Retention time	6.5 min
Tailing factor	1.196
Theoretical plates	9865

Table 5: Recovery data of paclitaxel

% of recovery	Formulation concentration	Spiked concentration	Total concentration	Concentration obtained	% recovery
50	4	2	6	5.95	99.17
				5.93	98.83
				5.98	99.66
				Mean	99.22
100	4	4	8	7.98	99.75
				8.05	100.62
				7.96	99.00
				Mean	99.79
150	4	6	10	10.17	101.70
				10.03	100.30
				9.97	99.70
				Mean	100.56

Table 6: Precision study of paclitaxel

Components	Intraday precision				Interday precision			
	Retention time		Peak area		Retention time		Peak area	
	Mean	%RSD	Mean	%RSD	Mean	%RSD	Mean	%RSD
2	6.47	0.73	18248.66	0.041	6.46	0.85	18252.33	0.044
4	6.51	0.93	49893.33	0.007	6.54	0.95	49899	0.0106
6	6.53	0.85	67317.66	0.010	6.54	0.89	67319.66	0.011

Table 7: Limit of detection and limit of quantification

Parameter	Paclitaxel
LOD(µg/mL)	0.09809
LOQ(µg/mL)	0.2942

Table 8: System suitability parameters

Parameters	Obtained Values
Peak area	107286
Theoretical plates (N)	10274
Tailing factor (T)	1.297
Asymmetric factor	1.1

Table 9: Robustness results of paclitaxel

Condition		Tailing factor	Theoretical plates	%RSD
Optimized condition		1.256	10447	-
Mobile phase ratio	**55:45**	1.109	9856	0.980
(50:50)	**45:55**	1.323	10219	1.102
Flow rate(mL/min)	**1.1**	1.156	9648	1.035
(1.0 mL/min)	**0.9**	1.208	10065	0.998
pH of phosphate buffer	**4.4**	1.006	9963	1.121
(4.5)	**4.6**	1.149	9896	0.975
Wavelength(nm)	**281**	1.345	10159	1.142
(282)	**283**	1.216	10278	1.069

Accuracy

Accuracy, which is the measure of closeness of the experimental value to the true value, was determined by standard addition method. To a pre-analyzed sample formulation a known quantity of standard was added at three levels (50, 100 and 150% of the assay concentration). The experiment was performed in triplicates. The % recoveries were calculated for all the concentrations. Results are summarized in Table 5.

Precision

Method Precision was determined in terms of repeatability (intra-day) and intermediate precision (inter-day) studies by measuring the peak area and retention time of 3 different concentrations (2, 4 and 6 µg mL-1) of paclitaxel. Repeatability was performed by repeated injections of 3 different concentrations from single batch under the same experimental conditions on the same day. Intermediate precision of the method was evaluated by performing the analysis on three different days for three different concentrations of paclitaxel. From the results, RSD values for retention time were <1%, while RSD values for peak area were <2% for both intra-day and inter-day assay precision**.** Precision results are expressed in Table 6.

Sensitivity

Sensitivity of the method was determined from limit of detection (LOD) and limit of quantitation (LOQ). The LOD and LOQ were determined using the calibration curve and results are summarized in Table 7.

$$LOD = 3.3 \times D/S \text{ and } LOQ = 10 \times D/S,$$

Where,

D = standard deviation of y intercept of regression line

S = slope of the calibration curve

System suitability tests

The test was carried out by making six replicate injections of a standard solution containing 6 µg/mL of paclitaxel and analyzing each solute for their peak area, theoretical plates (*N*), tailing factor (*T*), and asymmetric factor (As). System suitability parameters are tabulated in Table 8.

Robustness

Robustness of the method was studied to evaluate the effect of small but deliberate variations of the chromatographic conditions on the method parameters. Robustness was determined by changing individually the flow rate (1.0 ± 0.1 mL/min), organic solvent (50 ± 5%) and ionic strength of buffer (4.5 ± 0.1). Their effects on tailing factorand theoretical plates were studied and tabulated in Table 9.

CONCLUSION

The UFLC method developed was accurate, precise, reproducible and specific. The method is economical and utilizes a mobile phase which can be easily prepared. The method is less time consuming. All these merits make this method suitable for quantification of paclitaxel in bulk and pharmaceutical dosage forms without interference.

ACKNOWLEDGEMENTS

The authors express their sincere thanks to the Principal, JSS College of Pharmacy, Mysore and JSS University, Mysore for providing the necessary facilities to carry out the research work.

CONFLICT OF INTEREST

No conflict of interest.

ABBREVIATIONS USED

UFLC: Ultra-Fast Liquid Chromatography; **HPLC:** High Performance Liquid Chromatography; **LC-MS:** Liquid Chromatography-Mass spectrophotometry; **ICH:** International Conference of Harmonization; **RSD:** Relative standard deviation.

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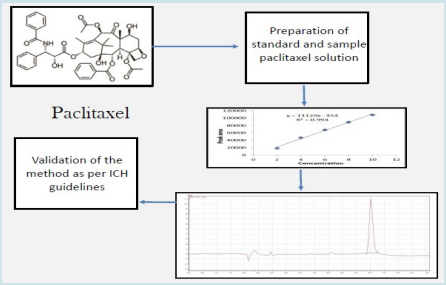
PICTORIAL ABSTRACT



SUMMARY

- Simple, sensitive, precise and rapid RP-UFLC method for the analysis paclitaxel in pure and pharmaceutical dosage form was developed.
- The developed method was validated according to current ICH guidelines.

ABOUT AUTHORS

Manusmitha Sriram Srinivasan: Is a post graduate student in the Department of Pharmaceutical chemistry at the JSS college of Pharmacy, JSS University, Mysuru. Her research area of interests are analytical method development and validation for synthetic and pharmaceutical formulations.

Ravandur Shivanna Chandan: Currently working as an Assistant Professor in the Department of Pharmaceutical chemistry at the JSS College of Pharmacy, JSS University, Mysuru. He has obtained his Ph.D. Degree in 2013 from Dr. M G R University, Chennai. His main research interests are in the areas of Analytical and Bio-analytical method development for the pharmaceutical drugs and Impurity profiling.

Venkata Sairam Koganti: Is a Research scholar at JSS University; JSS College of Pharmacy Mysuru, Where he graduated in Bachelor and Master of Pharmacy. His main research interests are in the areas of Analytical and Bio-analytical method developments for the pharmaceutical drugs and its validation. He has published widely in international journals and conferences.