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Analytical Method Development and Validation of Paclitaxel Pharmaceutical Dosage Forms by RP-HPLC

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ABSTRACT

A simple, precise, accurate RP-HPLC method was developed and validated for the estimation of Paclitaxel in pharmaceutical dosage forms. An Agilent column (4.6×150mm) 5µ, flow rate was 1.0 ml/min, mobile phase ratio was Methanol: Phosphate buffer (60:40% v/v), detection wavelength was 226nm. The instrument used was WATERS HPLC Auto Sampler, Separation module 2695, photo diode array detector 996, Empower-software version-2. The retention times were found to be 4.687 mins. The % purity of Paclitaxel was found to be 98.56%.The system suitability parameters for Paclitaxel such as theoretical plates and tailing factor were found to be 3797331, 1.1. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study of Paclitaxel was found in concentration range of 20µg-100µg and correlation coefficient (r2) was found to be 0.999, % recovery was found to be 98.96%, %RSD for repeatability was 0.05 % RSD for intermediate precision was 0.87. The precision study was precision, robustness and repeatabilty.LOD value was 3.01 and LOQ value was 9.58. The results obtained in the study were within the limits of ICH guidelines and hence this method can be used for the estimation of Paclitaxel in pharmaceutical dosage forms.

Keywords: Agilent column, Paclitaxel, RP-HPLC

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1. Introduction

Paclitaxel (PTX) sold under the brand name Taxol among others, is a chemotherapy medication used to treat a number of types of cancer. This includes ovarian cancer, breast cancer, lung cancer, Kaposi sarcoma, cervical cancer, and pancreatic cancer. It is given by injection into a vein. There is also an albumin-bound formulation. Common side effects include hair loss, bone marrow suppression, numbness, allergic reactions, muscle pains, and diarrhea. Other serious

side effects include heart problems, increased risk of infection, and lung inflammation. There are concerns that use during pregnancy may cause birth defects. Paclitaxel is in the taxane family of medications. It works by interference with the normal function of microtubules during cell division.

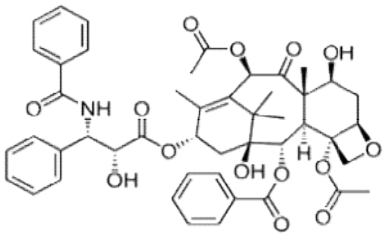

Fig 1: Chemical structure of Paclitaxel

2. Materials and Method

Materials:

Paclitaxel, KH2PO4, Water and Methanol for HPLC, Acetonitrile for HPLC, Ortho phosphoric Acid.

Instrumentation:

HPLC-auto sampler –UV detector, Separation module2695, UV.detector2487, Empower-software version-2 Waters. U.V double beam spectrophotometer LABINDIA, UV 3000+pH meter, Weighing machine.

Chromatographic conditions:

Column : Agilent (4.6×150mm)5µ
Mobile phase ratio : Methanol: Phosphate buffer (60:40% v/v)
Detection wavelength : 226 nm
Flow rate : 1.0 ml/min
Injection volume : 10µl
Column temperature : Ambient
Auto sampler temperature : Ambient
Run time : 10min
Retention time : 4.687min

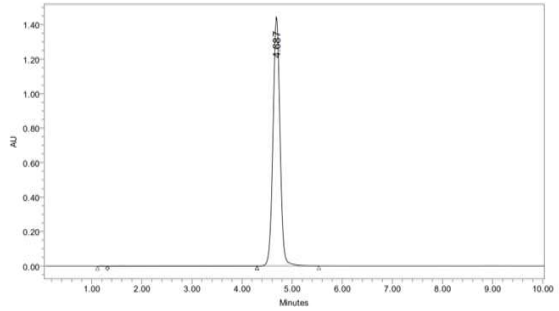

Fig 2: Optimized Chromatogram

Observation:

The separation was good, peak shape was good, so we conclude that there is no required for increse the retention times of peak, so it is taken as final method.

Sample solution preparation:

10 mg of Paclitaxel tablet powder was accurately weighed and transferred into a 10 ml clean dry volumetric flask, add about 2ml of diluent and sonicate to dissolve it completely and making volume up to the mark with the same solvent(Stock solution). Further pipette 10ml of the above stock solution into a 100ml volumetric flask and was diluted up to the mark with diluent.

Standard solution preparation:

10 mg Paclitaxel working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).Further pipette out 1ml of the above stock solution into a 10ml volumetric flask and was diluted up to the mark with diluent.

Method Validation

Specificity:

The system suitability for specificity was carried out to determine whether there is any interference of any impurities in retention time of analytical peak. The specificity was performed by injecting blank.

Linearity:

10 mg of Paclitaxel working standard was accurately weighed and was transferred into a 10ml clean dry volumetric flask, add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

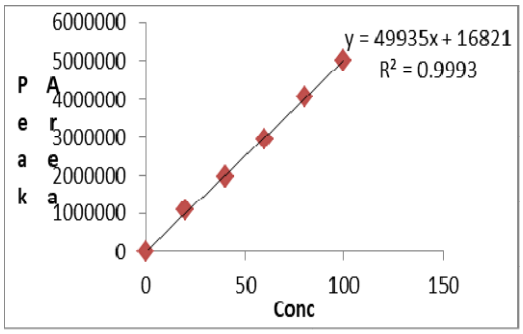

Fig 3: Calibration graph of Paclitaxel

Range:

Based on precision, linearity and accuracy data it can be concluded that the assay method is precise, linear and accurate in the range of 20µg/ml-100µg/ml of Paclitaxel.

Accuracy:

10mg of Paclitaxel working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).

Precision:

10 mg of Paclitaxel working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Intermediate Precision/Ruggedness:

To evaluate the intermediate precision (also known as ruggedness) of the method, precision was performed on different days by using different make column of same dimensions.

Limit of detection (LOD): LOD's can be calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (S) at levels approximating the LOD according to the formula. The standard deviation of the response can be determined based on the standard deviation of y-intercepts of regression lines.

Limit of quantification:
LOQ's can be calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (S) according to the formula. Again, the standard deviation of the response can be determined based on the standard deviation of y-intercepts of regression lines.

Robustness:
As part of the robustness, deliberate change in the flow rate, mobile phase composition was made to evaluate the impact on the method.

System suitability: 10 mg of Paclitaxel working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent

3. Results and Discussion

Table 1: Linearity Results for Paclitaxel

S.No	Linearity Level	Concentration	Area
1	I	20	1083048
2	II	40	1973321
3	III	60	2955166
4	IV	80	4063921
5	V	100	5006038
Correlation Coefficient			0.999

Table 2: Accuracy results for Paclitaxel

%Concentration (at specification level)	Average area	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
50%	3162304	5	4.86	98.81%	98.96%
100%	6172922	10	9.88	99.08%	
150%	9382075	15	15.0	100.0%	

Table 3: Precision results for Paclitaxel

	Name	RT	Area
1	Paclitaxel	3.515	2947505
2	Paclitaxel	3.516	2950484
3	Paclitaxel	3.515	2950895
4	Paclitaxel	3.517	2949444
5	Paclitaxel	3.512	2951420
Mean			2949950
Std.Dev.			1547.3
%RSD			0.05

Table 4: Intermediate precision of Paclitaxel

	Name	RT	Area
1	Paclitaxel	3.517	2905047
2	Paclitaxel	3.514	2904166
3	Paclitaxel	3.517	2902300
4	Paclitaxel	3.517	2949444
5	Paclitaxel	3.512	2951420
Mean			2922475
Std.Dev.			25549.7
%RSD			0.87

Table 5: Results for Limit of Detection

Drug name	Standard deviation(σ)	Slope(s)	LOD(µg/ml)
Paclitaxel	1547	49935	3.01

Table 6: Results for Limit of Quantitation

Drug name	Standard deviation(σ)	Slope(s)	LOQ(µg/ml)
Paclitaxel	1547	49935	9.58

Table 7: Robustness results for Paclitaxel (Change in flow rate)

S. No	Flow rate (ml/min)	System suitability results	
		USP Plate Count	USP Tailing
1	0.8	2645	1.5
2	1	2530	1.5
3	1.2	2404	1.5

Table 8: Robustness results for Paclitaxel (Change in Mobile phase)

S. No	Change in organic composition in the mobile phase	System suitability results	
		USP Plate Count	USP Tailing
1	5 % less	2422	1.5
2	*Actual	2542	1.4
3	5 % more	2610	1.6

4. Conclusion

A simple, rapid, accurate, and precise HPLC analytical method has been developed and validated for the routine analysis of Paclitaxel in pharmaceutical dosage forms by RP-HPLC. The results of stress testing,under taken according to the ICH guidelines, Hence the suggested RP-HPLC method can be used for routine analysis of Paclitaxel in API and Pharmaceutical dosage form.

5. References

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