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Research

New Analytical Method Development And Validation For The Determination Of Paclitaxel In Bulk Drug And Formulation By Rp-Hplc

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Abstract

A rapid and precise Reverse Phase High Performance Liquid Chromatographic method has been developed for the validated of Paclitaxel, in its pure form as well as in tablet dosage form. Chromatography was carried out on a Symmetry C18 (4.6 x 250mm, 5µm) column using a mixture of Acetonitrileand Water (50:50% v/v)as the mobile phase at a flow rate of 0.8ml/min, the detection was carried out at 285nm. The retention time of the Paclitaxelwas 3.0±0.02min. The method produces linear responses in the concentration range of 10-50ppm of Paclitaxel. The method precision for the determination of assay was below 2.0%RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.

Keywords: Paclitaxel, RP-HPLC, validation.

INTRODUCTION

Quality can be definedasthecharacter, which defines the grade of excellence. Agoodquality drug is something, which will meet the established product specifications, can besafely bought and confidently used for the purpose for which it is intended.[1] To get a good qualitydrug, the manufacturing form a king a drug should hav equality built into it.

Analytical chemistryis the science that seeks ever improvedmeans of measuring thechemical composition of natural and artificial materials. Analytical chemistry is a sub-discipline of chemistry tha thast hebroad mission of understanding the chemical composition of all matter and developing the tools to elucidate such compositions.[2]

Types
Traditionally, analytical chemistry has beensplit into two main types, qualitative andquantitative.

Qualitative
Qualitative analysis seeks to establish the presence of agivenelement, given functional group or inorganic or organic compound in a sample.

Quantitative
Quantitative analysis seeks to establish the amount of a given element or compound in asample.

Modern analytical chemistry
Modern analytical chemistry is dominated by instrumental analysis. There are so many different types of instruments today that it can seem like a confusing array of acronyms rather than a unified field of study. Most modern analytical chemistry is categorized by different analytical methods.

Analytical methods
- Spectrophotometry and colorimetry.
- UV-visible spectroscopy.
- Chromatography and Electrophoresis.

Commonly used method sare
- High Performance Liquid Chromatography(HPLC).
- High Performance Thin Layer Chromatography(HPTLC).
- Gaschromatography(GC).
- Gaschromatography-Massspectroscopy(GC-MS).
- Liquid chromatography-Massspectroscopy(LC-MS).

Meaning of chromatography and its type
Chromatography is a versatile analytical technique used to separate, identify, and quantify components in a mixture. It is based on the differential distribution of the components between two phases: a stationary phase and a mobile phase. As the mixture passes through the stationary phase while being carried by the mobile phase, the components interact differently with the stationary phase, leading to their separation. Chromatography is a method used for separating organic and inorganic compounds so thatthey can be analysed and studied. Chromatography is a great physical method for observingmixtures and solvents. The word chromatography means colour separation where chromameans colour and graphy means separation. Chromatography is based on different migration. Solutes with a greater affinity for the mobile phase will spend more time in this phase than solutes that prefer the stationary phase. As the solutes move through the stationary phase the different components are going to be absorbed and are going to stop moving with mobilephase. Thus they are separated. Thisis called as chromatographic development.

The different type of chromatography[7]
Adsorption chromatography

Adsorption chromatogra phyisprobably one of the oldest types of chromatography around. Itutilises a mobile liquid or gaseous phase that is absorbed on to the surface of a stationarysolid phase. The equilibrium between the mobile and stationary phase accounts for theseparationofdifferentsolutes.

Partition chromatography
This form of chromatography is based on thin film formed on the surface of a solid support by a liquid stationary phase. Solutes equilibrates between the mobilephase and the stationary liquid.

Ionexchange chromatography
In this type of chromatography, the use of a resin (the stationary solid phase) is used tocovalently attach anions or cations to it. Solute ions of the opposite charge in the mobile liquidphase are attracted to the resin by electrostatic forces.

Molecular exclusion chromatography

Also known as gel permeation or gel filtration, this type of chromatography lacks an attractive interaction between stationary phase and solute. The liquid or gaseous phase passes through a porous gel, which separates the molecule according to its size. The pores are normally small and exclude the larger solute molecule, but allow smaller molecule to enter the gel, causing them to flow through a larger volume. This causes the larger molecules to passthrough the columnata faster rate than the smallerones.

Affinity chromatography

This is the most selective type of chromatography employed. It utilizes the specificin teraction between one kind of solute molecule and a second molecule that is immobilisedon a stationary phase. For example the immobilised molecule may be an antibody to somes pecific protein. When solutecon taining a mixture of proteinis passed by this molecule,only the specific proteinis reacted to this antibody, it to the binding stationary phase. This protein is later extracted by changing the ionic strength or PH.

Highperformanceliquidchromatography

HPLC is able to separate macromolecules and ionic species labile natural products, polymericmaterials, and awide variety of other high–molecularweightpolyfunctionalgroup.HPLCisthe fastest growing analytical technique for the analysis of the drugs. It's simplicity, high specificity, and wide range of sensitivity makes it ideal for the analysis of many drugs in both dosage forms and biological fluids. In this, the separation is about 100 times faster than the conventional liquid chromatography due to packing of particles in the range of 3-10µm. Modern LC uses very small particles for packing. The small particle size results in more rapid approach to the distribution equilibrium and consequently smaller plate height, so that a given length of column includes large number of plates which makes the column efficient and the peak narrow. But close packing of these smal lparticles reduces the flow rate of the mobile phase through the packed bed (the packing said to develop high back pressure) and in order to achieve a reasonable flow rate it is necessary to apply pressure to the mobile phase. So the designation, put forth as high pressure liquid chromatography. Thus HPLC ishaving advantages of improved resolution, faster separation, improved accuracy, precision and sensitivity.

According to the phases involved, HPLC can be classified into several types which is asfollows

1. Normal phase chromatography (NPC)
2. Reverse–Phase chromatography (RPC)
3. Liquid–solid chromatography oradsorption HPLC
4. Liquid-liquid chromatography orpartition HPLC
5. Ion exchange chromatography orion exchange HPLC
6. Size exclusion orgelpermeationorsteric exclusion HPLC
7. Ionpair HPLC
8. Affinity HPLC

Normal Phase Chromatography(NPC)

In normal phase chromatograph, the stationary phase is more polar than the mobile phase,and the mobile phase is a mixture of organic solvents without added water (eg.Isopropane with hexane) and the column packing is either an inorganic adsorbent (silica) or a polarbonded phase (cyano, diol,amino) on a silica support. Sample retention in normal phase chromatography increases as the polarity of the mobile phase decreases. They are eluted in the order of increasing polarities.

Reversephase chromatography

Inreverse phase chromatography, the stationary phaseis less polar than the mobile phase and the mobile phaseisa mixture of organi can daqueousphase. Reverse phase chromatography is typically more convenientan drugged than the other forms of liquid chromatography and is more likely to result in a satisfactory final separation. High Performance RPC columns areefficients table and reproducible. In this the solutes are eluted in the order of their decreasing polarities. These are prepared by treating the surface silanol group of site with an organicchlorosilanere agent.

MATERIALS AND METHODS

Paclitaxel (Pure)-Sura labs, Water and Methanol for HPLC-LICHROSOLV (MERCK), Acetonitrile for HPLC-Merck, Potassium Dihydrogen Phosphate-Finar Chemicals.

Hplc method development
Trails
Preparation of Standard Solution

Accurately weigh and transfer 10 mg of Paclitaxel working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol. Further pipette 2ml of the above Paclitaxel stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Mobile Phase Optimization

Initially the mobile phase tried was methanol: Water and ACN: Water with varying proportions. Finally, the mobile phase was optimized to Acetonitrile: Phosphate Buffer (25:75% v/v) respectively.

Optimization of Column

The method was performed with various C18columns like Symmetry, Zodiac and Xterra. Phenomenex Luna C18 (4.6mm x 150mm, 5µm) was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

Optimized chromatographic conditions:

Instrument used	:	Waters HPLC with auto sampler and PDA996 detector model.
Temperature	:	Ambient
Column	:	Phenomenex Luna C18 (4.6mm x 150mm, 5µm)
Mobile phase	:	Acetonitrile: Phosphate Buffer (pH-2.8) (25:75% v/v)
Flow rate	:	1.0mL/min
Wavelength	:	220 nm
Injection volume	:	10 µl
Run time	:	8 minutes

Preparation of mobile phase
Preparation of mobile phase

Accurately measured 250 ml of Acetonitrile (25%) and 750 ml (75%) Phosphate Buffer (pH-2.8) were mixed and degassed in a digital ultrasonicator for 15 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation

The Mobile phase was used as the diluent.

RESULTS AND DISCUSSION

Optimized Chromatogram (Standard)

Column	: Symmetry C18 (4.6×250mm) 5µ
Column temperature	: Ambient
Wavelength	: 285nm
Mobile phase ratio	: Acetonitrile:Water(50:50 v/v)
Flow rate	: 0.8ml/min
Injection volume	: 10µl
Run time	: 6minutes

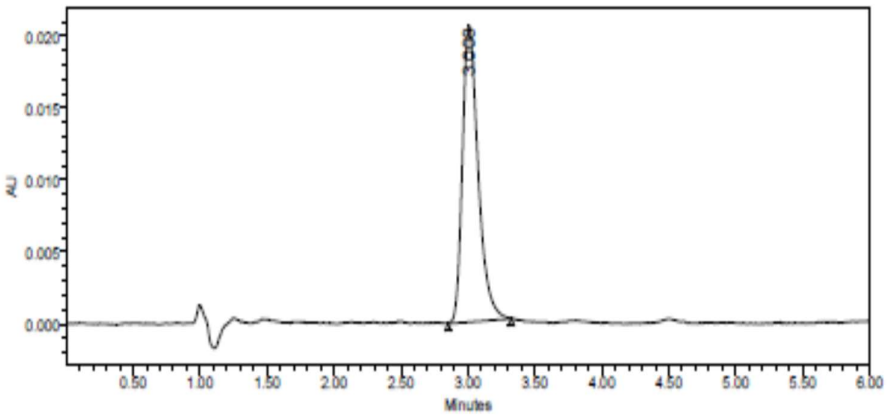


Fig 1: Optimized Chromatogram (Standard)

Table 1: Optimized Chromatogram (Standard)

S.no	Name	RT	Area	Height	USPTailing	USPPlate Count
1	Paclitaxel	3.008	627611	41541	1.1	9917

Optimized Chromatogram (Sample)

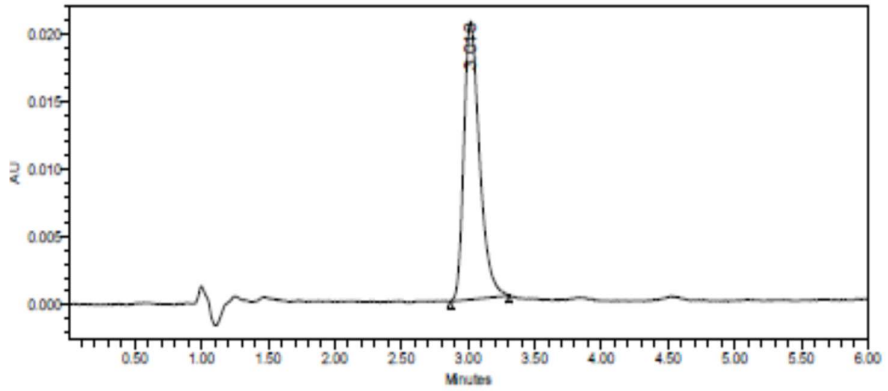


Fig 2: Optimized Chromatogram (Sample)

Table 2:Optimized Chromatogram (Sample)

S.no	Name	RT	Area	Height	USPTailing	USPPlate Count
1	Paclitaxel	3.018	738718	27711	1.2	8937

Theoretical plates must be not less than 2000, Tailing factor must be not less than 0.9 and not more than 2.
It was found from above data that all the system suitability parameters for developed method were within the limit.

System suitability

Table 3: Results of system suitability for Paclitaxel

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Paclitaxel	2.982	168830	20736	4726	1.1
2	Paclitaxel	2.988	168066	20583	7781	1.2
3	Paclitaxel	3.001	168565	20647	9173	1.1
4	Paclitaxel	3.008	168387	20631	5534	1.2
5	Paclitaxel	3.018	168406	20545	5526	1.1
Mean			168450.8			

Std.Dev.	278.8292
%RSD	0.165526

%RSD of five different sample solutions should not more than 2
The %RSD obtained is within the limit, hence the method is suitable.

Assay (Standard)

Table 4: Peak results for assay standard

S.No	Name	RT	Area	Height	USPTailing	USPPlateCount	Injection
1	Paclitaxel	2.804	163305	21223	1.2	5817.1	1
2	Paclitaxel	2.865	163387	20960	1.1	6144.5	2
3	Paclitaxel	2.988	168830	20736	1.1	6452	3

Assay (Sample)

Table 5: Peak results for Assay sample

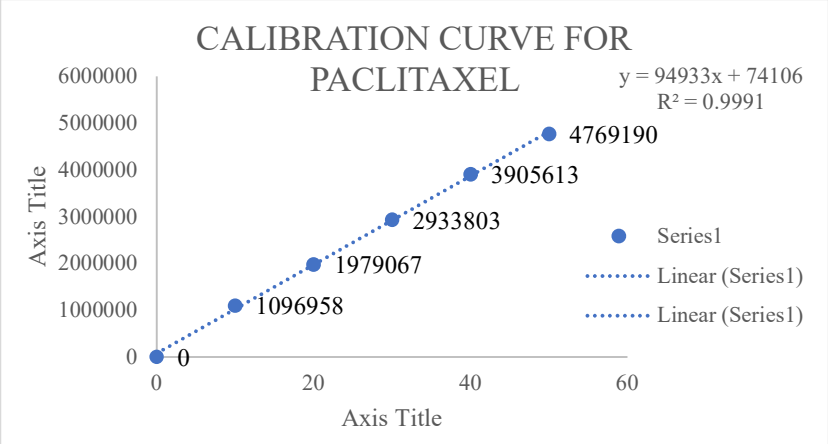
S.No	Name	RT	Area	Height	USPTailing	USPPlateCount	Injection
1	Paclitaxel	2.804	162052	28151	1.1	8862	1
2	Paclitaxel	2.865	163387	21761	1.2	7872	2
3	Paclitaxel	3.018	168406	21956	1.1	6926	3

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

=99.5%
The % purity of Paclitaxel in pharmaceutical dosage form was found to be 99.5%.

Linearity
Chromatographic data for linearity study

Concentration Level (%)	Concentration µg/ml	Average Peak Area
60	10	1096958
80	20	1979067
100	30	2933803
120	40	3905613
140	50	4769190



Repeatability

Table 6: Results of repeatability for Paclitaxel

S. No	Peak name	Retention time	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Paclitaxel	2.942	168306	20744	7562	1.1
2	Paclitaxel	2.962	168388	20788	9981	1.2
3	Paclitaxel	2.963	168365	20727	6794	1.1
4	Paclitaxel	2.804	162052	21841	8927	1.2
5	Paclitaxel	2.865	163387	21947	7746	1.1
Mean			166099.6			
Std.dev			3121.629			
%RSD			1.879372			

%RSD for sample should be NMT 2

The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

Intermediate precision

Day-1

Table 7: Results of Intermediate precision for Paclitaxel

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Paclitaxel	2.982	167483	20736	8846	1.21
2	Paclitaxel	2.988	167196	20583	9173	1.1
3	Paclitaxel	3.001	167072	20647	8892	1.2
4	Paclitaxel	3.008	167281	20631	9937	1.1
5	Paclitaxel	3.018	167508	20545	9946	1.2
6	Paclitaxel	3.018	168406	21452	8817	1.1
Mean			167491			
Std.Dev.			478.3856			
%RSD			0.285619			

%RSD of six different sample solutions should not more than 2

Day 2:

Table 8: Results of Intermediate precision Day 2 for Paclitaxel

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP PlateCount	USP Tailing
1	Paclitaxel	2.982	168830	20736	9371	1.1
2	Paclitaxel	2.988	168066	20583	8916	1.2
3	Paclitaxel	3.001	168565	20647	7917	1.1
4	Paclitaxel	3.008	168387	20631	9971	1.1
5	Paclitaxel	3.018	168406	20545	7977	1.2
6	Paclitaxel	3.018	167508	20655	6018	1.1
Mean			168293.7			
Std.Dev.			458.6304			
%RSD			0.272518			

%RSD of six different sample solutions should not more than 2

Accuracy

Table 9:The accuracy results for Paclitaxel

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	86774	15	14.9	99.8	99.6
100%	168427	30	29.79	99.3	
150%	255311	45	44.8	99.7	

The percentage recovery was found to be within the limit (98-102%).

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

Robustness

Table 10: Results for Robustness

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 0.8 mL/min	168461	3.008	8846	1.12
Less Flow rate of 0.7mL/min	167261	4.608	7927	1.1
More Flow rate of 0.9mL/min	167651	3.495	6927	1.1
Less organic phase (about 5 % decrease in organic phase)	168947	4.609	8826	1.2
More organic phase (about 5 % Increase in organic phase)	160081	3.499	9971	1.1

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Paclitaxel in bulk drug and pharmaceutical dosage forms. This method was simple, since diluted samples are directly used without any preliminary chemical derivatization or purification steps. Paclitaxel was freely soluble in acetonitrile ethanol, methanol and sparingly soluble in water. Water: Acetonitrile (50:50% v/v) was chosen as the mobile phase. The solvent system used in this method was economical. The %RSD values were within 2 and the method was found to be precise.The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods. This method can be used for the routine determination of Paclitaxel in bulk drug and in Pharmaceutical dosage forms.

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