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Development and Validation of Hplc Method For Analysis of Paclitaxel Drug

Manoj Phadtare[1]*, Prof Smita Aher[1], Dr. R.S. Bachhav[1], Dr. Dipti G. Phadatre[1], Dr. Anita Patil[2]

[1]KCT 's R.G Sapkal College of Pharmacy, Anjaneri, Nashik, Maharashtra, India

[2]Head of Department, Distance Education BVDU, YMIM, Karad, Maharashtra, India

Corresponding Author

Manoj Phadtare;

KCT 's R.G Sapkal College of Pharmacy, Anjaneri, Nashik, Maharashtra, India

Email Id: mnpharma1@gmail.com

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ABSTRACT

A high-performance liquid chromatography method was established for the quantification of related components in an intravenous emulsion containing a paclitaxel–cholesterol combination. The separation was accomplished via an Agilent Luna® 100 A° C8 (150 X 4.6 mm, 5 µm), maintained at 20C°. The gradient mobile phase comprised acetonitrile and water, with a flow rate of 1.2 ml/min. The ultraviolet detection wavelength was established at 230 nm. The sample solution preparation commenced with the incorporation of anhydrous sodium sulphate to disrupt the emulsion. Methanol and ethyl ether were subsequently introduced to extract the medication and eliminate the emulsion's components using extraction and centrifugation. The method demonstrated selectivity, sensitivity, robustness, linearity, repeatability, accuracy, and appropriateness for quantifying paclitaxel-related substances in emulsion formulations, with the primary degradation products.

Keywords: *Development, Validation, HPLC Method, Analysis and Paclitaxel Drug.*

1. INTRODUCTION

Paclitaxel (PAC) is a diterpenoid pseudoalkaloid that was extracted in the early 1960s from the bark of the Pacific Yew. It exhibits unique antitumor efficacy and has been effectively employed to treat several cancers, including refractory ovarian cancer, metastatic breast cancer, non-small cell lung cancer, head and neck malignancies, and AIDS-related Kaposi's sarcoma. Owing to its limited solubility in water, numerous generic PAC-based formulations, in addition to the original patented PAC, Taxol, are presently prepared as a 50/50 (v/v) blend of Cremophor EL and pure ethanol. Cremophor EL is linked to significant adverse effects and induces hypersensitivity. As to AHFS Drug Information, a premedication protocol for steroids is often initiated up to 12 hours prior, whereas H-receptor antagonists should be administered 30-60 minutes before PAC to mitigate the risk of severe hypersensitivity reactions. This formulation is linked to several concerns, including stability, potential drug precipitation upon dilution, filtration needs, and the necessity for non-plasticized containers and delivery sets (1, 2).

Figure 1 Structure of Paclitaxel

In the last 15-20 years, numerous studies have concentrated on enhancing drug solubility, which is essential for intravenous administration. To accomplish this objective, one may utilise cosolvents, alter the PAC molecule into prodrugs or analogues, and construct liposomes or micelles. Nevertheless, none of these formulations has been implemented in clinical practice thus far, owing to inadequate biocompatibility to satisfy the standards for intravenous preparations. The inaugural product exhibiting a superior clinical profile compared to Taxol is a nanosuspension of PAC coupled with human albumin (Abraxane®). Despite its superior clinical profile, Abraxane is not typically supplanting Taxol in oncology, primarily due to its elevated cost. Therefore, alternative and economical parenteral formulations of PAC remain necessary (3, 4). Our laboratory has recently created a novel PAC o/w emulsion (PACE) encapsulating a PAC–cholesterol complex. The results indicate that such PACE demonstrates enhanced stability, capable of withstanding complete heat sterilisation and prolonged storage. It demonstrates superior biocompatibility and safety for intravenous infusion based on animal experiments. Moreover, given that the PACE consisted entirely of clinically acceptable excipients and was produced using standard high-pressure homogenisation, it was appropriate for industrial-scale manufacturing and clinical use. To optimise the formulation, assess the stability, and regulate the quality of the prospective emulsion product, it was essential to establish a method for determining the PAC-related chemicals in PACE (5, 6). The majority of analytical techniques documented in the literature for the separation of PAC utilised high-performance liquid chromatography (HPLC) and focused on identifying related chemicals in plant extracts, raw materials, and Taxol formulations. Limited approaches have been documented in detail for the PACE. The primary challenge faced during HPLC technique development was the inclusion of many excipients in the emulsion formulations, including plant oil, emulsifier, coemulsifier, and osmotic regulator. The creation of emulsion sample solutions is essential for the quantitative examination of emulsions. Prior research has indicated a straightforward technique that entails directly diluting the material with an organic solvent, necessitating substantial quantities of the solvent. Nevertheless, given that PACE samples were introduced at a PAC concentration of 0.8-1.2 mg/ml, the solvent dilution approach cannot achieve a sufficiently high concentration of PAC for the identification of associated chemicals (7, 8). Furthermore, various excipients in the emulsions frequently absorb ultraviolet (UV) light, potentially causing interferences in the quantification of the medicine and its related components. Moreover, if emulsion samples are not pre-processed, the column may be compromised due to the accumulation of oils and the impact of surfactants upon injection into the HPLC. An effective demulsification procedure has been demonstrated to eliminate the oils. Demulsification can be accomplished through the addition of chemical demulsifiers, including acids, bases, and salts, as well as by centrifugation. Nonetheless, the incorporation of demulsifiers is not appropriate in every instance, and it may even compromise the efficacy of specific pharmaceuticals (9, 10). Currently, there is no information on how to maintain a constant ratio of associated compounds to the medicine during the emulsion breaking process. Moreover, following the disruption of the emulsion, the extraction of lipophilic medicines from the oils becomes challenging. The medicine can typically be isolated from the oils only when its solubility in oils markedly differs from that in organic solvents. Consequently, the choice of appropriate organic solvents and extraction methods is crucial for sample preparation. The aim of this work was to design a sample solution preparation procedure and to build a validated HPLC method for the quantification of relevant chemicals in PACE. All validation parameters were executed, encompassing specificity, robustness, linearity, accuracy, precision, limit of detection (LOD), and limit of quantification (LOQ). Chromatographic separation was conducted using a Luna® 100 A° C8 column with a gradient mobile phase of acetonitrile and water. This approach is appropriate for identifying related chemicals of PAC in the novel pharmaceutical product (11, 12).

2. MATERIALS AND METHOD

Reagent and chemicals:

All reagents and solvents were analytical and HPLC grades, except Formic acid (Rankem, India) and Ammonium Acetate (Rankem, India). The water used was distilled and deionised by using Millipore (ELIX) system. Paclitaxel of the highest grade (purity>98.0%) were used as the external standards. In which Paclitaxel e were procured as gift sample from Fresenius Kabi Oncology Ltd., Kolkata, West Bengal.

Instrumentation and Chromatographic conditions:

Instruments used were mentioned in Table 1.

Table 1 Instrumentation

Instruments	Model No.	Manufacturer
HPLC	1260 Infinity II	Agilent
HPLC Column	Eclipse Plus C18 (150mm x 4.6mm, 5µm)	Agilent

Detector	Photo Diode Array	-
UV-Visible Spectrophotometer	UV- 1900	Shimadzu
PH meter	EQ-610	Lab Line
Ultra Sonicator	LMUC 6	-
Water purification system	-	Mili- Q
Analytical Weighing Balance	ME204/A04	Shimadzu

RP-HPLC method development for the estimation of paclitaxel in bulk drug and formulations with forced degradation studies (13-16)

Trial 1

Table 2 Chromatographic conditions:

Instrument	:	HPLC
Column	:	Phenomenax LUNA SCX 4.6 X 50 mm, 5 µm
Injection Volume	:	20 µL
Flow rate	:	2.0 mL/min
Wavelength	:	UV 230 nm
Column Temperature	:	30°C
Sample Temperature	:	25°C
Retention Time	:	About 3.0 minute for paclitaxel
Run Time	:	20 minutes Standard and SST
	:	40 minutes for Blank, Placebo and sample
Needle wash	:	Mixture of Acetonitrile and Water in the ratio of 90:10.
Seal wash	:	Mixture of Acetonitrile and Water in the ratio of 10:90.

Preparation of solutions:

Dilute Orthophosphoric acid:

Transfer 1 mL of Orthophosphoric acid into 1000 mL volumetric flask containing about 200 mL of water and mix well. Cool and dilute with water to volume and mix.

Buffer solution pH-3.5:

Weigh accurately about 6.8 g of monobasic potassium phosphate and transfer into 1 litter of purified water and stir well to dissolve. Adjust the pH to 3.5 ± 0.05 with dilute Orthophosphoric acid under stirring. Stop the stirring, wait for about 10 minutes and confirm the pH, if require then adjust the pH of buffer solution. Filter the solution through 0.45 µm PVDF membrane filter.

Mobile phase:

Prepare a mixture of buffer solution and Acetonitrile.in the ratio of 85:15. Sonicate the solution for 15 minutes to degas.

Dilute Orthophosphoric acid for diluent preparation:

Transfer 2.0 mL of Orthophosphoric acid into 2000 mL volumetric flask containing about 200 mL of water and mix well. Cool and dilute with water to volume and mix.

Diluent:

Prepare a mixture of diluted phosphoric acid and Acetonitrile in the ratio of 95:5. Sonicate for 10 minutes to degas.

Trial 2

Buffer, gradient program, column and column oven temperature Study to develop new methodology for Assay test of paclitaxel.

Table 3 Chromatographic conditions:

Instrument	:	HPLC
Column	:	ACE EXCEL 250X 4.6 mm, 3 µm
Injection Volume	:	20 µL
Flow rate	:	1.0 mL/min
Wavelength	:	UV 230 nm
Column Temperature	:	40°C
Sample Temperature	:	25°C

Preparation of solutions:

Buffer solution pH-2.5:

Weigh accurately about 2.0 g of Tetrabuyl ammonium hydrogen sulphate and transfer into 2 litter of purified water and stir well to dissolve (Observed pH 2.547). Filter the solution through 0.45 µm PVDF membrane filter.

Mobile phase:

Prepare a mixture of buffer solution and Acetonitrile in the ratio of 95:05. Sonicate the solution for 15 minutes to degas.

Trial 3

Buffer, gradient program, column and column oven temperature Study to develop new methodology for Assay test of paclitaxel.

Table 4 Chromatographic conditions:

Instrument	:	HPLC
Column	:	ACE EXCEL 250X 4.6 mm, 3 µm
Injection Volume	:	5 µL
Flow rate	:	0.6 mL/min
Wavelength	:	UV 230 nm
Column Temperature	:	25°C
Sample Temperature	:	25°C

Preparation of solutions:

Buffer solution pH-2.5:

Weigh accurately about 8.0 g of Tetrabuyl ammonium hydrogen sulphate and transfer into 2 litter of purified water,2.0mL Triethylamine and stir well to dissolve (Observed pH 2.532). Filter the solution through 0.45 µm PVDF membrane filter.

Mobile phase A:

Used Buffer pH 2.532

Mobile phase B:

Used 100% Acetonitrile.

Trial 4

Buffer, gradient program, column and column oven temperature Study to develop new methodology for Assay test of paclitaxel.

Table 5 Chromatographic conditions:

Instrument	:	HPLC
Column	:	ACE EXCEL 250X 4.6 mm, 3 µm
Injection Volume	:	5 µL
Flow rate	:	0.8 mL/min
Wavelength	:	UV 230 nm
Column Temperature	:	50°C
Sample Temperature	:	25°C

Preparation of solutions:

Dilute Orthophosphoric acid for buffer pH adjustment preparation:

Transfer 10.0 mL of Orthophosphoric acid into 100 mL volumetric flask containing about 200 mL of water and mix well. Cool and dilute with water to volume and mix

Buffer solution pH-2.5:

Weigh accurately about 5.0 g of 1-Heptane sulfonic acid salt and transfer into 2 litter of purified water, and adjusted pH to 2.472 with diluted Orthophosphoric acid. Filter the solution through 0.45 µm PVDF membrane filter.

Mobile phase A:

Used Buffer pH 2.472

Mobile phase B:

Used 100% Acetonitrile.

Trial 5

Table 6 Chromatographic conditions:

Mode	:	HPLC
Column	:	Luna® 100 A° C8, 150 X 4.6 mm, 5 µm (or) equivalent
Injection Volume	:	10 µL
Flow rate	:	1.5 mL / minute
Wavelength	:	UV 215 nm
Column oven Temperature	:	20°C
Sample Temperature	:	25°C

Retention Time	:	About 5.5 Minutes
Run Time	:	20 Minutes
Needle wash	:	Mixture of Acetonitrile and Water in the ratio of 90:10.
Seal wash	:	Mixture of Acetonitrile and Water in the ratio of 10:90.

Preparation of solutions:

Blank (Diluent):

Prepare a mixture of Acetonitrile: Water in the ratio of 20:80 and degas by sonication.

Buffer solution:

Dissolve 2.72 g Potassium dihydrogen phosphate and 1.0 g 1-Octane sulfonic acid sodium salt into 1000 mL of water. Filter through 0.22 µm PVDF membrane filter under constant stirring.

Mobile phase A:

Prepare a mixture of buffer solution and acetonitrile in the ratio of 80:20. Sonicate for 10 minutes to degas.

Mobile phase B:

Prepare a mixture of buffer solution and acetonitrile in the ratio of 30:70. Sonicate for 10 minutes to degas.

RP-HPLC method Validation for the estimation of Paclitaxel in bulk drug and formulations with forced degradation studies

Table 7 Chromatographic conditions:

Mode	:	HPLC
Column	:	Luna® 100 A° C8, 150 X 4.6 mm, 5 µm (or) equivalent
Injection Volume	:	10 µL
Flow rate	:	1.5 mL / minute
Wavelength	:	UV 230 nm
Column oven Temperature	:	20°C
Sample Temperature	:	25°C
Retention Time	:	About 5.5 Minutes
Run Time	:	20 Minutes
Needle wash	:	Mixture of Acetonitrile and Water in the ratio of 90:10.
Seal wash	:	Mixture of Acetonitrile and Water in the ratio of 10:90.

3. METHODOLOGY (TEST PROCEDURE):

Preparation of solutions:

Blank (Diluent):

Prepare a mixture of Acetonitrile: Water in the ratio of 20:80 and degas by sonication.

Buffer solution:

Dissolve 2.72 g Potassium dihydrogen phosphate and 1.0 g 1-Octane sulfonic acid sodium salt into 1000 mL of water. Filter through 0.22 µm PVDF membrane filter under constant stirring.

Mobile phase A:

Prepare a mixture of buffer solution and acetonitrile in the ratio of 80:20. Sonicate for 10 minutes to degas.

Mobile phase B:

Prepare a mixture of buffer solution and acetonitrile in the ratio of 30:70. Sonicate for 10 minutes to degas.

Standard stock solution:

Weigh accurately about 48 mg of working standard / primary reference standard into 500 mL volumetric flask; add about 300 mL of diluent. Sonicate for about 5 minutes and ensure standard gets dissolved completely and cool at room temperature, dilute with diluent to volume and mix well.

Sample solution: (Prepare sample in duplicate)

Take 5 ampoules of Paclitaxel injections, transfer the all solution in one 50 mL test tube. Mixed well and Pipette out 4 mL of clear solution and transfer into 250 mL volumetric flask, add about 170 mL of diluent. Sonicate for 45 minutes with intermittent shaking. Allow to cool at room temperature and dilute with diluent to volume and mix well. Filter the portion of solution through 0.45 µm nylon syringe filter discarding first 2-3 mL filtrate. Use clear filtrate as a sample solution.

Procedure:

Equilibrate the HPLC column with mobile phase at least one hour and then condition with one complete gradient program. Separately inject 10 µL of blank, standard solution and sample solution into the chromatograph as per injection sequence given in table below. Record the chromatograph and measure the peak area response for Paclitaxel.

Table 8 Injection sequence

Sr. No.	Sample name	No. of injections
1	Blank	1
2	Standard solution	6
3	Sample solution_1	1
4	Sample solution_2	1
5	Standard solution (Bracketing)	1

4. VALIDATION PROCEDURE:

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present in the sample matrix. Typically these might include impurities, degradants, matrix, etc. (17)

Linearity

The linearity of an analytical method is its ability to elicit test results that are directly or by well-defined mathematical transformation proportional to the concentration of an analyte in sample (18).

Accuracy

The accuracy of an analytical method is the closeness of the test result obtained by that method to the true value. The accuracy may often to be expressed as percent recovery of known amount of analyte added. Accuracy is a measure of the exactness of the analytical method that is true for all practical purposes (19).

Precision

The precision of an analytical method is the closeness of agreement between series of measurements obtained from multiple samplings of the same homogeneous sample under the prescribed condition (20).

Range

The range of an analytical procedure is the interval between the upper and lower concentration of analyte in the sample for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity. The range is normally expressed in the same units as test results obtained by analytical method (21).

Solution stability

Prepare the standard and sample solution as per methodology and store at room temperature. Analyse the solution at different

time interval and evaluate the results. For standard solution calculate overall % RSD of peak area of Paclitaxel and for sample solution compare the % assay values obtained at different time intervals (22).

Robustness

Make deliberately below changes in the chromatographic conditions, one by one and observe their effect on the system suitability test and % assay value. To evaluate robustness of the method, prepare the sample solution for Paclitaxel 20 mg Injection as per methodology. Analyse the sample solution for assay through chromatographic conditions recommended in test procedure with following method variables (23).

System suitability

System suitability shall be performed at the beginning of every validation parameter. Check the system suitability as per methodology (24).

Forced degradation study

The forced degradation study will be carried out on placebo, Paclitaxel API and Paclitaxel 30 mg injections. The samples will be subjected for acid degradation, base degradation, oxidation degradation, hydrolysis degradation, photolytic degradation, humidity degradation and thermal degradation. For each degradation study prepare a blank accordingly (25).

5. RESULT AND DISCUSSION

RP-HPLC method development for the estimation of paclitaxel in bulk drug and formulations with forced degradation studies.

Trial 1

Observations-

1 At tailing of main peak hump observed.

2. Theoretical plate count, tailing factor for main peak not good.

6. CONCLUSION-

Buffer, gradient, column and column oven temperature Study needs to be performed.

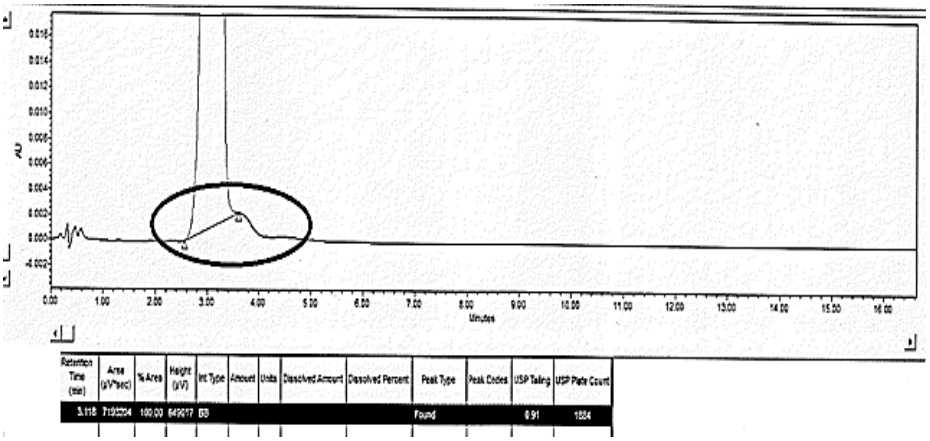

Figure 2 Chromatogram for Trial 1-

Trial 2

Observations

1 .At tailing of main peak baseline pattern not good

7. CONCLUSION

Buffer, gradient, column and column oven temperature Study needs to be performed.

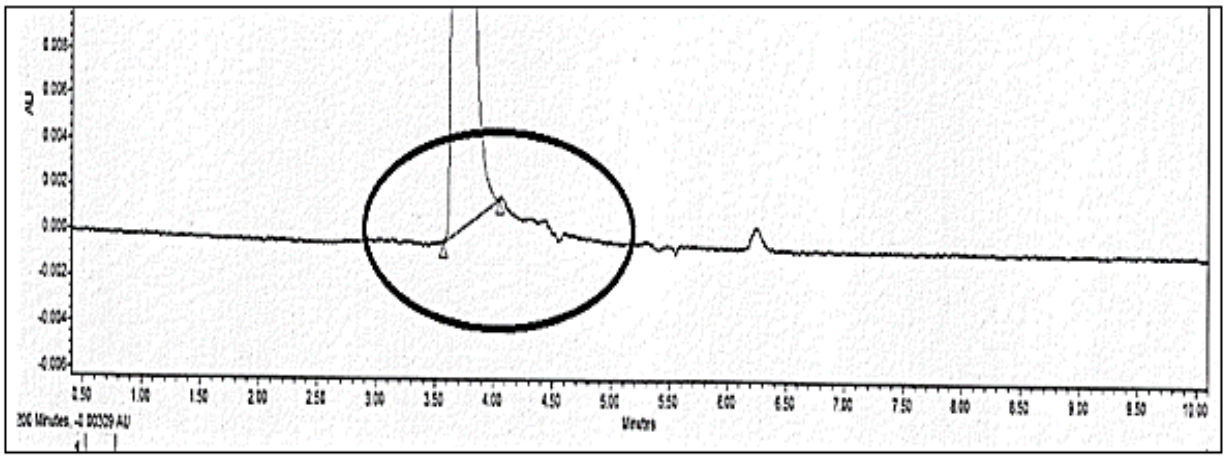

Figure 3 Chromatogram for Trial 2

Trial 3

Observations-

At tailing of main peak baseline pattern not good

Conclusion-

Buffer, gradient, column and column oven temperature Study needs to be performed.

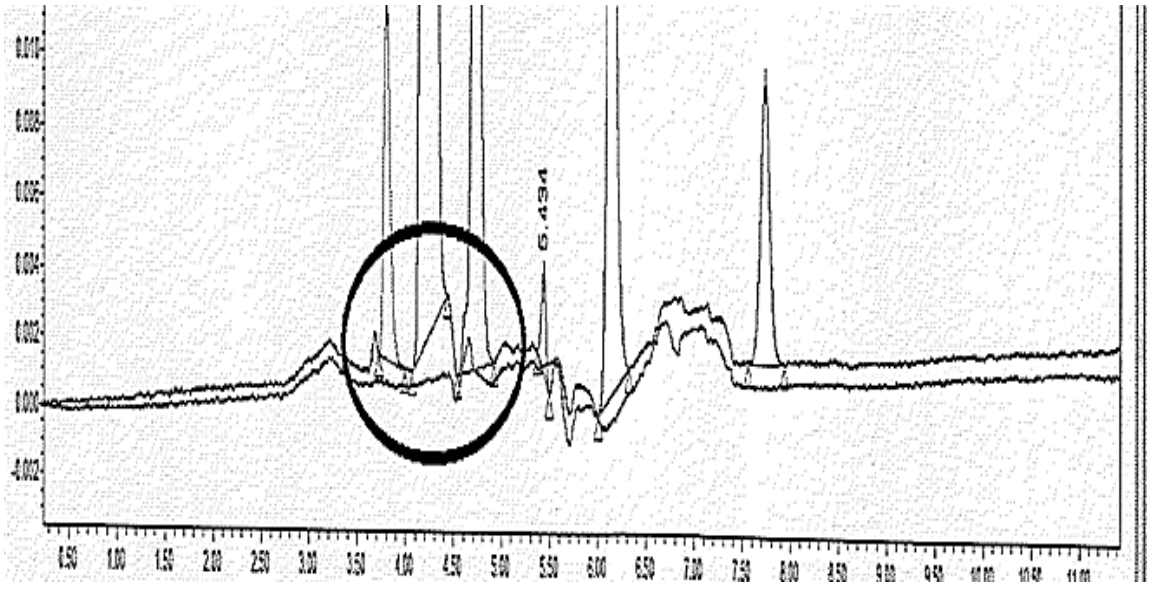

Figure 4 Chromatogram for Trial 3-

Trial 4

Observations-

1. Tailing of main observed about 1.64 which is slightly higher.
2. All impurities peaks are well separate from main peak.

8. CONCLUSION-

To improve tailing gradient, column oven temperature and injection volume study needs to be performed.

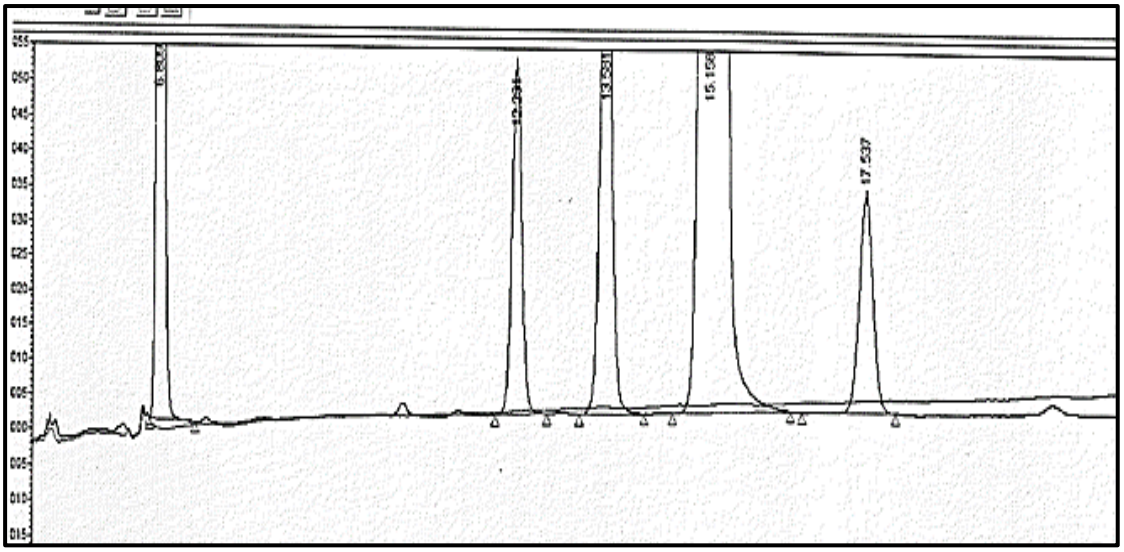

Figure 5 Chromatogram for Trial 4-

Trial 5

Observation:

1. No interference Blank peak at retention time of main peak impurities peaks.
2. All impurities peaks are well separate from each other's.
3. Tailing factor for main peak is about 5(improved compared with previous trial result).

9. CONCLUSION-

From observation this methodology can be finalized and validation study needs to be performed.

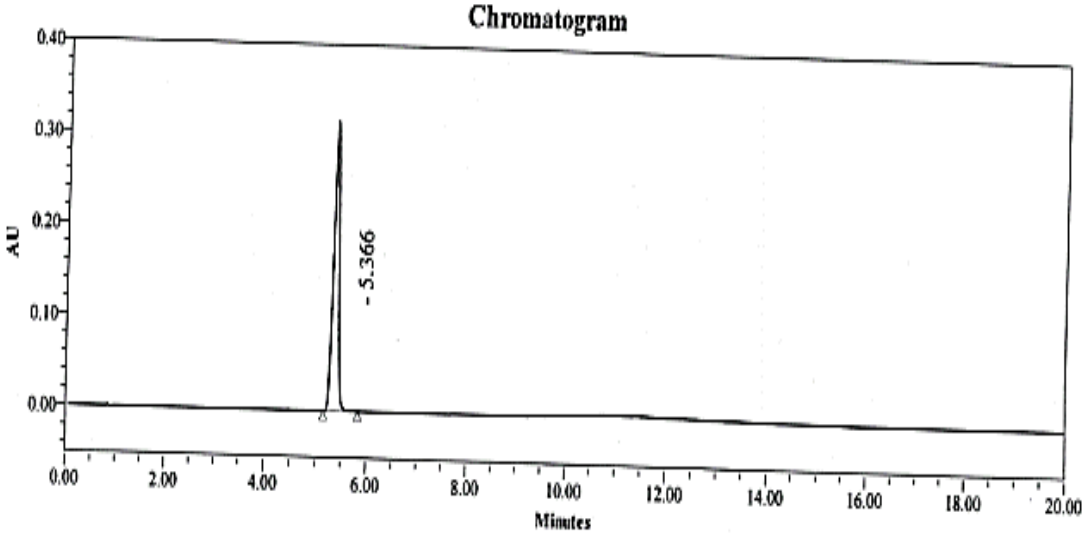

Figure 6 Chromatogram for Trial 5-

RP-HPLC method Validation for the estimation of paclitaxel in bulk drug and formulations with forced degradation studies.

Table 9 Validation Summary

Sr. No.	Validation Parameter	Results	Acceptance Criteria
10.1	Specificity		
10.1.1	Check for blank, placebo and impurities interference		
	Interference	There is no any interference observed due to blank, placebo and impurities at the retention time of Paclitaxel peak.	There should not be any interference due to blank, placebo and impurities at the retention time of Paclitaxel peak.
	Peak purity	Peak purity criteria (Peak purity = purity angle < purity threshold) passes for Paclitaxel peak in the standard solution, sample solution, placebo spiked with known impurity and Paclitaxel and sample spiked solution with known impurities.	Peak purity criteria (Peak purity = purity angle < purity threshold) should pass for Paclitaxel peak in the standard solution, sample solution, placebo spiked solution with known impurities and Paclitaxel and sample spiked solution with known impurities.
10.1.2	Forced degradation: Check for blank, placebo and degradation products interference		
	Interference	There is no any interference observed due to blank, placebo and degradation products with the Paclitaxel peak	There should not be any interference due to blank, placebo and degradation products with the Paclitaxel peak.
	Peak purity	Peak purity criteria (Peak purity = Purity angle < Purity threshold) passes for Paclitaxel peak in all the degraded samples.	Peak purity criteria (Peak purity = Purity angle < Purity threshold) should pass for Paclitaxel peak in all the degraded samples.

10.2	Linearity	Correlation coefficient ('R'): 0.99997				The correlation coefficient ('R') value should not be less than 0.99 over the working range.
10.3	Accuracy	Level	% Conc.	Mean % Recovery	% RSD	Individual and mean recovery for Paclitaxel should be between 98.0% to 102.0%. Overall mean recovery for Paclitaxel should be between 98.0% to 102.0%. Overall % RSD for Paclitaxel should not be more than 2.0%.
		1	50	101.2	0.2	
		2	100	100.9	0.2	
		3	150	100.4	0.3	
		Overall % Recovery		100.8		
		Overall % RSD		0.4		
10.4	Precision					
10.4.1	System precision	Tailing factor = 1.0				Tailing factor: Tailing factor of Paclitaxel peak obtained from 1st injection of standard solution should not be more than 2.0.

		Theoretical plates = 12990 % RSD = 0.1	Theoretical Plates: Theoretical plates of Paclitaxel peak obtained from 1st injection of standard solution should not be less than 2000. RSD: Relative standard deviation of Paclitaxel peak area obtained from six replicate injections of standard solution should not be more than 2.0%.

10.4	Precision (Continued…….)		
10.4.2	Method precision	% Assay of Paclitaxel 30 mg Injection: Sample 1 = 99.3 Sample 2 = 98.9 Sample 3 = 98.8 Sample 4 = 99.3 Sample 5 = 99.6 Sample 6 = 98.9 Mean % assay = 99.1 RSD of six determinations = 0.3%	Individual and mean % assay value should be within specification limit. The RSD of six determinations should not be more than 2.0%.
10.4.3	Intermediate precision	% Assay of Paclitaxel 30 mg Injection: Sample 1 = 99.5 Sample 2 = 99.3	Individual and mean % assay value should be within specification limit.

		Sample 3 = 99.2 Sample 4 = 98.0 Sample 5 = 99.0 Sample 6 = 98.6 Mean % assay = 98.9 RSD of six determinations = 0.6% Absolute difference = 0.2	The RSD of six determinations should not be more than 2.0%. Absolute difference between mean % assay values obtained from the method precision and intermediate precision study should not be more than 2.0.

10.5	Range	The established range for Paclitaxel assay method is 50% to 150% with respect to test concentration based on the validation data from linearity, accuracy and precision.	Range should be established based on the validation data from linearity, accuracy and precision.
10.6	Filter study	Absolute difference of % assay value obtained with centrifuged sample solution and sample solution filtered through, 0.45 µm nylon syringe filter = 0.0 0.45 µm pre-filter + PVDF syringe filter = 2.4* 0.45 µm pre-filter+ PTFE syringe filter = 0.1 *Note: 0.45 µm pre-filter + PTFE syringe filter get hard during filtration of sample solution.* **: Results does not meet acceptance criteria.*	The absolute difference of % assay value between centrifuged sample solution and filtered sample solution should not be more than 2.
10.7	Solution Stability	Standard Solution: Overall relative standard deviation of Paclitaxel peak area in the standard solution up to 78 hours at room temperature = 0.7% Sample Solution: Absolute difference in the % assay value of Paclitaxel obtained in sample solution at initial and at 50 hours at room temperature = 1.6	Overall relative standard deviation of Paclitaxel peak area in the standard solution obtained at different time interval should not be more than 2.0%. Absolute difference in the % assay value of sample solution obtained at the initial and after each time intervals should not be more than 2.0.
10.8	Robustness		

	Change in column oven temperature (+ 5°C) of 20°C	High column oven Temperature (HCT): 25°C Tailing Factor = 1.0 Theoretical plates = 13634 RSD = 0.1% RSD for sample solution = 0.3% Overall RSD = 0.4%	Tailing factor: Tailing factor of Paclitaxel peak obtained from 1st injection of standard solution should not be more than 2.0. Theoretical Plates: Theoretical plates of Paclitaxel peak obtained from 1st injection of standard solution should not be less than 2000. RSD: Relative standard deviation of Paclitaxel peak area obtained from six replicate injections of standard solution should not be more than 2.0%. RSD of % assay results obtained from three sample solutions with each altered condition should not be more than 2.0%. Overall RSD of % assay results obtained from method precision and each altered condition should not be more than 2.0%.
	Change in quantity of Potassium dihydrogen phosphate (± 10%) of 2.72 g	High Buffer (HB): 2.99 g Tailing Factor = 1.0 Theoretical plates = 12893 RSD = 0.1% RSD for sample solution = 0.5% Overall RSD = 0.4%	
		Low Buffer (LB): 2.45 g Tailing Factor = 1.0 Theoretical plates = 12604 RSD = 0.4% RSD for sample solution = 0.4% Overall RSD = 0.3%	

Validation Conclusion

The method was found specific with respect to determination of assay of Paclitaxel in Paclitaxel 30 mg Injection.

The method was found specific, linear, accurate, precise and rugged.

The standard solution is stable up to 78 hours and sample solution is stable up to 50 hours at room temperature.

0.45 µm nylon syringe filter is found suitable for filtration of sample solution.

The method is robust with respect to change in Column oven temperature (+ 5°C) of 20°C and change in quantity of Potassium dihydrogen phosphate (± 10%) of 2.72 g.

The values obtained for all validated parameters are well within the predefined acceptance criteria. Therefore, the analytical method is valid and suitable for its intended use to determination of Assay, Content uniformity and Blend uniformity of Paclitaxel in Paclitaxel 30 mg Injection.

10. CONCLUSION

The findings achieved by the suggested method for the determination of Paclitaxel via RP-HPLC are dependable, accurate, and exact. The standard deviation figures were deemed satisfactory, and the recovery studies approached 100%. The approach does not necessitate the prior separation of one medication from another. The attributes of the system, including precision, linearity, accuracy, robustness, and stability, were deemed satisfactory in accordance with ICH requirements. The proposed method was straightforward, precise, time-efficient for sample analysis, and easy to execute. HPLC exhibits superior separation efficiency relative to HPTLC. Nonetheless, HPTLC can yield rapid qualitative results and achieve high-resolution separation with precision and accuracy comparable to that of HPLC and GC. Currently, HPTLC is regarded as a

dependable analytical technique for the quantitative study of micro, nano, and even pictogram levels in complicated formulations. HPTLC is an off-line process wherein multiple steps are executed individually. This configuration with an open, disposable layer offers significantly higher throughput and reduced cost per study compared to an online column procedure like HPLC (26-28).

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