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# Development and Validation of a Robust RP-HPLC Method for the Quantification of Paclitaxel: A Comprehensive Study in Pharmaceutical Analysis

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**ABSTRACT :**
This study presents the development and validation of a reverse phase high-performance liquid chromatography (RP-HPLC) method for the accurate quantification of paclitaxel, a potent chemotherapeutic agent. The method employs acetonitrile : methanol (60:40) as the mobile phase, a Symmetry C18 column, and UV detection at 227nm. Validation was conducted following International Conference on Harmonization (ICH) guidelines, encompassing linearity, accuracy, precision, robustness, and limit of detection(LOD) and limit of quantification(LOQ).The RP-HPLC method exhibits exceptional linearity, with a correlation coefficient ($r^2$) of 0.999, ensuring a consistent relationship between paclitaxel concentration and peak area. Accuracy assessments demonstrate recoveries within the range of 98.58% to 99.33%, validating the method's capability to accurately determine paclitaxel concentrations. Precision studies reveal low percentage relative standard deviation (%RSD) values, indicating the method's reliability and stability. Robustness tests confirm the method's resilience to minor variations in flow rate, acetonitrile, and methanol concentrations. The determined LOD and LOQ at 1.57µg/ml and 4.76µg/ml, respectively, underscore the method's sensitivity, crucial for its application in pharmaceutical formulations and clinical research .The developed RP-HPLC method, characterized by its simplicity, accuracy, and sensitivity, proves to be a valuable analytical tool for routine paclitaxel quantification in pharmaceutical formulations and biological matrices. This research contributes to advancing analytical methodologies in pharmaceutical analysis, ensuring the accurate determination of paclitaxel, a crucial chemotherapeutic agent.

**KEYWORDS:** Reverse phase high-performance liquid chromatography, Paclitaxel, Chemotherapeutic agent, validation, pharmaceutical analysis

## I. INTRODUCTION

Taxol, a renowned brand in cancer therapeutics, owes its efficacy to paclitaxel, a potent chemotherapeutic agent derived from the bark of the Pacific Yew Tree (Taxus brevifolia). Discovered in 1967, paclitaxel has been a cornerstone in the treatment of various cancers, demonstrating significant success in clinical trials spanning several decades [1].Its approval by the US Food and Drug Administration (FDA) in 1992 for advanced ovarian cancer marked a pivotal moment in cancer treatment. Subsequent approvals for metastatic breast cancer, Kaposi's sarcoma, and non-small cell lung cancer underscored paclitaxel's versatility.[2]

However, paclitaxel's characteristics pose challenges. As a crystalline powder with low water solubility, its oral bioavailability is limited, necessitating parenteral formulations. Traditional formulations like Taxol® present challenges such as hypersensitivity reactions and rapid bloodstream clearance. Innovations like Abraxane®, a colloidal suspension of paclitaxel in human albumin, address some issues but come with elevated costs and limited efficacy[3].The chemical complexity of paclitaxel necessitates precise analytical methods for its determination. High-performance liquid chromatography (HPLC) has been a stalwart in this pursuit due to its sensitivity and versatility. However, the existing landscape of HPLC methodologies for paclitaxel analysis exhibits

variations in extraction techniques, sensitivity, and adherence to validation standards.[4]

This study addresses this gap by introducing a novel RP-HPLC method for paclitaxel determination. With a focus on simplicity and resilience, this method aims to streamline the analytical process while ensuring robust and reliable results. The emphasis on adherence to International Council for Harmonisation (ICH) guidelines for method validation adds a layer of credibility, reinforcing the method's suitability for pharmaceutical and clinical research applications. In a broader context, the introduction of this RP-HPLC method contributes to the ongoing efforts to optimize paclitaxel analysis. By combining simplicity, resilience, and adherence to international quality standards, this method aligns with the evolving needs of pharmaceutical research and development, promising advancements in cancer treatment and patient care.

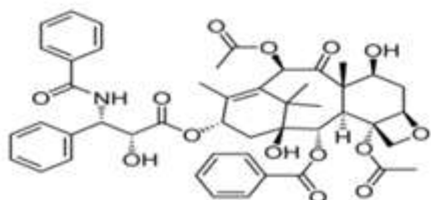


Figure 1 : Chemical Structure of Paclitaxel

## II.HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Various analytical methodologies have been extensively employed to establish robust assays for the identification of paclitaxel in bodily fluids. Among these techniques, capillary electrophoresis, chromatographic-mass spectrometry (LCMS), immunoassays, and high-performance liquid chromatography (HPLC) stand out. The versatility of HPLC methods has made them particularly prominent in pharmacokinetic and toxicokinetic investigations of paclitaxel, owing to their inherent high sensitivity and straightforward applicability. In the realm of HPLC, a variety of techniques are employed, each with its unique approach to sample preparation and extraction. Solid-phase extraction (SPE), protein precipitation combined with SPE, liquid-liquid extraction (LLE), and hybrid methodologies combining SPE and solvent extraction are commonly employed in paclitaxel analysis[5].While certain techniques utilize intricate extraction procedures, others may lack the required sensitivity or fail to meet validation standards set forth by the International Conference on Harmonisation (ICH).High-performance liquid chromatography (HPLC) techniques have been integral to the investigation of paclitaxel since the 1980s, reflecting their established efficacy in pharmaceutical analysis[6]. This study introduces a novel approach, outlining a straightforward, resilient, and expeditious RP-HPLC technique for the determination of paclitaxel. The method's emphasis on simplicity and resilience makes it a valuable addition to the existing analytical toolkit for paclitaxel. Crucially, the accuracy, precision, limit of detection (LOD), and limit of quantitation (LOQ) of this newly proposed RP-HPLC method have undergone rigorous validation in accordance with the International Council for Harmonisation (ICH) guidelines. This validation process ensures that the method meets the highest standards for reliability and robustness in the quantification of paclitaxel. By aligning with ICH guidelines, the study emphasizes the importance of adherence to international quality standards in analytical methodologies, ensuring the credibility and reproducibility of results. The presented RP-HPLC technique, with its validated parameters, contributes to advancing the field of pharmaceutical research and development, promising accurate and dependable analysis of paclitaxel in diverse applications.[7]

## III.MATERIALS AND METHODS

### INSTRUMENTS

The HPLC system (Waters Corporation) consist of Waters 2489 UV/Vis Detector, Pump A and B (515 HPLC Pump),Symmetry C18 5µm (4.6 X 250mm) column,20µl Loop,25µl Injector and Empower 2 Software was used for data analysis.

### CHEMICALS AND REAGENTS

Paclitaxel was purchased from Moleculochem Private Limited (Ahmedabad, Gujarat). Acetonitrile and Methanol from Merck Life Science (Bengaluru, Karnataka) and HPLC water grade were obtained from Atlas Chemicals (Pune, Maharashtra).

### CHROMATOGRAPHIC CONDITIONS AND DETECTION PARAMETERS

The present study investigates the chromatographic conditions and detection parameters utilised in the experiment.

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The analytical column utilised for the separation was a Symmetry C18 5µm (4.6 x 250mm), while the detection was carried out using a Waters 2489 UV/Vis Detector. The mobile phase employed was a mixture of Acetonitrile and Methanol in a ratio of 60:40. The experimental protocol involved the use of a 20µL injection volume and elution was conducted at a flow rate of 1ml/min, with UV detection at a wavelength of 227nm. The separations were executed at ambient temperature while adhering to the optimised parameters [8].

## PREPARATION OF STANDARD SOLUTIONS

The preparation of standard solutions involved the dissolution of accurately weighed quantities of paclitaxel (100µg/ml) in a mixture of Acetonitrile and Methanol in a ratio of 60:40. Subsequently, the solution underwent filtration using a 0.2 millipore filter and was subjected to High Performance Liquid Chromatography (HPLC). Various concentrations of Paclitaxel standard ranging from 10µg/ml to 50µg/ml were prepared for the purpose of validating the method.

## METHOD VALIDATION

The validation of the method has been carried out in accordance with the International Council for Harmonisation (ICH) guidelines, encompassing a range of parameters including linearity, accuracy, precision, limit of detection (LOD), limit of quantitation (LOQ), and robustness. The primary objective of validating an analytical method is to establish its fitness for the intended purpose.

## LINEARITY

The analytical method's linearity refers to its capacity to yield test outcomes that are directly proportional to the analyte concentration in the sample, despite variations in data such as amount and AUC. The determination of linearity was conducted through the examination of standard concentrations of paclitaxel (ranging from 10 to 50 µg/ml) that were derived from a stock solution. The analysis was performed at a wavelength of 227nm, utilising Acetonitrile: Methanol (60:40) as the mobile phase.[4]

## ACCURACY

As per the guidelines outlined in ICH Q2 (R1), the accuracy of an analytical method is indicative of the level of concurrence between a value that is acknowledged as a true conventional value or a recognised reference value and the value that is obtained.

The percentage recovery method was employed to ascertain accuracy. The percentage of recoveries was ascertained for three established concentrations of Paclitaxel standard, namely 20, 40, and 60µg/ml. The procedure was replicated thrice for every concentration.[4]

## PRECISION

As per the ICH Q2 (R1) guidelines, the precision of an analytical method is indicative of the level of concurrence among a set of measurements procured from diverse samplings of an identical homogeneous specimen, while adhering to predetermined conditions. The concept of precision can be observed through three distinct levels, namely repeatability, intermediate precision, and reproducibility.

The method's precision was evaluated through the determination of its repeatability and intermediate precision. The determination of intermediate precision was conducted with regards to the reproducibility of intra-day and inter-day measurements. The analysis of repeatability was conducted through the execution of six replicates of identical concentration set at 20 µg/ml. The measurement of the quantity was carried out at three distinct time points on the same day, specifically, intra-day and conducted intermediate precision using a comparable methodology for three distinct occasions (inter-day).[4]

## LOD AND LOQ

The Limit of Detection (LOD) and Limit of Quantification (LOQ) are important parameters in analytical chemistry.

The definition of Limit of Detection (LOD) is the concentration at which the signal-to-noise ratio reaches 3. The determination of the Limit of Quantification (LOQ) was performed by establishing the minimum concentration that could be detected at a signal-to-noise ratio of 10.

The limits of detection (LOD) and quantification (LOQ) were evaluated through the utilisation of standard paclitaxel solutions. Paclitaxel solutions of standard concentration were formulated and evaluated to determine their respective limits of quantification and detection. The limit of detection (LOD) and limit of quantification (LOQ) were ascertained.[9]

The equation for LOD = 3.3/Slope

The equation for LOQ =10/Slope

**ROBUSTNESS**

The capacity of a method to maintain its stability in the face of minor alterations is referred to as its robustness. The study conducted strength tests, wherein minor modifications were made to the flow rate, quantity of methanol and acetonitrile. The findings pertaining to the robustness of this approach indicated that there were no statistically significant alterations in the flow rate within the range of 12 ml/min to 1.4 ml/min and reported a fluctuation in the percentage of Methanol and Acetonitrile, ranging from 43% to 48%.[9]

## IV.RESULTS AND DISCUSSION

**CHROMATOGRAPHIC CONDITIONS AND DETECTION PARAMETERS**

An isocratic RP-HPLC/UV detection method was developed, the method employed Acetonitrile : Methanol(60:40) as the mobile phase, a flow rate of 1.5ml/min, a wavelength of 227nm, an injection volume of 20µl, and Symmetry C18 5µm(4.6 X 250 mm) as the column. The method was deemed simple and dependable. The proposed methodology is a High Performance Liquid Chromatography (HPLC) technique that is characterised by its expeditiousness, precision, accuracy, affordability, and efficiency in the qualitative and quantitative assessment of paclitaxel in pharmaceuticals. Additionally, this method is suitable for the determination of paclitaxel in biological matrices in the context of clinical research.

Table 1: Chromatographic conditions and detection parameters

| Detector | UV-Vis Detector |
|---|---|
| Column | SymmetryC18 5µm(4.6 ×250mm) column |
| Mobile Phase | Acetonitrile : Methanol(60:40) |
| Flow rate | 1.5ml/min |
| Wavelength | 227nm |
| Injection volume | 20µl |

**STANDARD SOLUTIONS**

Standard solutions refer to established and widely accepted methods or approaches for solving common problems or achieving specific goals. These solutions are typically based on best practises, industry standards, or proven methodologies, and are often used as a benchmark or reference point for evaluating alternative solutions. Standard solutions can provide a framework for decision-making and problem-solving, and can help to ensure consistency, efficiency, and effectiveness in various domains, such as business, engineering, healthcare, or education.

Various concentrations of standard solutions were prepared, ranging from 20µg/ml to 100µg/ml. The construction of the paclitaxel standard curve was executed through the utilisation of Microsoft Excel. The coefficient of determination ($R^2$) was determined to be 0.999 through the utilisation of Microsoft Excel.

Table 2 : Standard curve of paclitaxel:

| Concentration (µg/ml) | Peak Area X $10^5$ |
|---|---|
| 20 | 6.4 |
| 40 | 11.2 |
| 60 | 16.6 |
| 80 | 21.5 |
| 100 | 26.7 |

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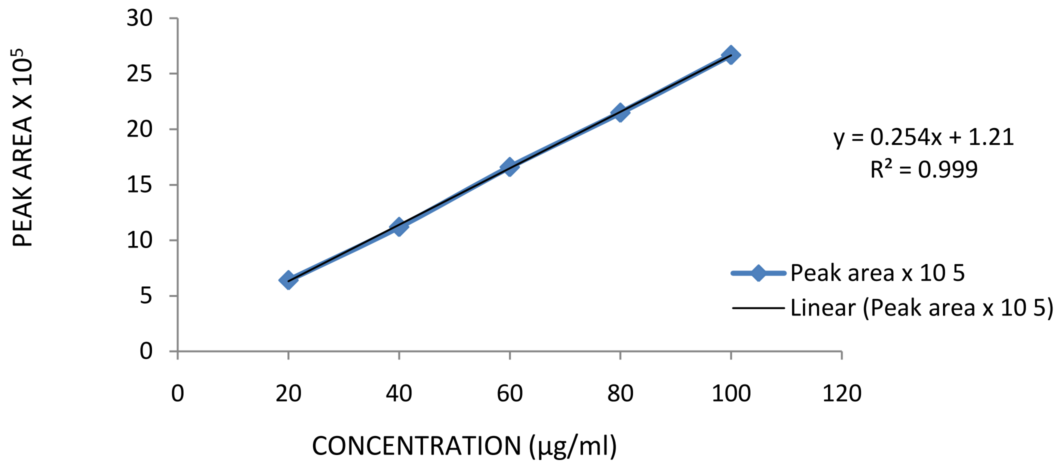


Figure 2: Standard curve of Paclitaxel using Microsoft Excel

**METHODVALIDATION**

**LINEARITY**

The validation of the linearity of the calibration curve was conducted through the utilisation of correlation coefficients ($r^2$) values. The obtained value was determined to be 0.999.

Table 3: Linearity table of paclitaxel:

| Concentration (µg/ml) | Peak Area X $10^5$ |
|---|---|
| 10 | 3.85 |
| 20 | 6.46 |
| 30 | 8.67 |
| 40 | 11.22 |
| 50 | 13.81 |

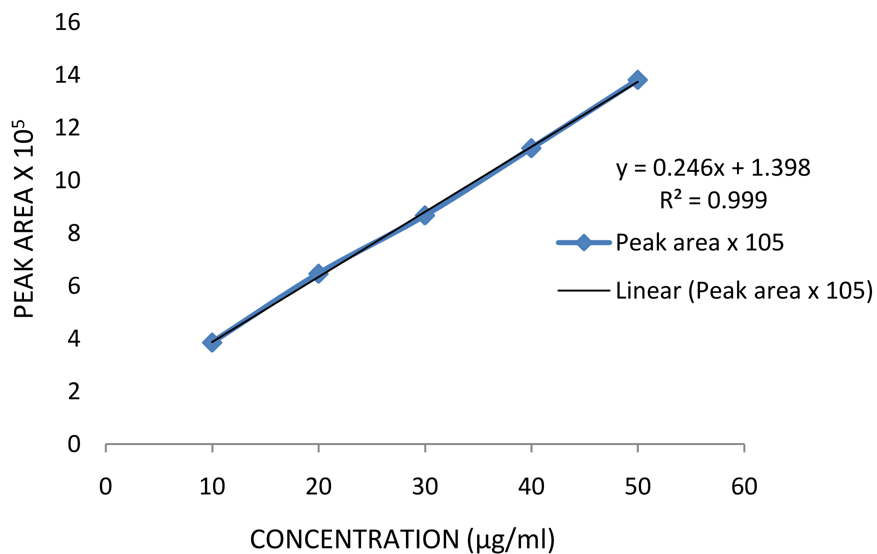


Figure 3: Calibration curve of paclitaxel (Linearity)

## ACCURACY

Accuracy was determined through standard addition and by analysing the outcomes of accuracy investigations. The method that was devised demonstrated a high level of accuracy, as evidenced by the percent recovery falling within the acceptable range of 90% to 110%, and the %RSD remaining below 2.

Table 4: Accuracy table of paclitaxel

| Sample No | Theoretical Concentration (µg/ml) | Measured Concentration (µg/ml) | %Recovery $(\frac{\text{Measured value}}{\text{Theoretical value}} \times 100)$ | %Recovery Mean | %RSD $(\frac{SD}{Mean} \times 100)$ |
|---|---|---|---|---|---|
| 1 | 20 | 20.2 | 101 | 99.33 | 1.5 |
| 2 | 20 | 19.6 | 98 | | |
| 3 | 20 | 19.8 | 99 | | |
| 4 | 40 | 39.7 | 99.25 | 98.58 | 1.8 |
| 5 | 40 | 40.0 | 100 | | |
| 6 | 40 | 38.6 | 96.5 | | |
| 7 | 60 | 59.2 | 98.66 | 99.21 | 1.4 |
| 8 | 60 | 60.5 | 100.83 | | |
| 9 | 60 | 58.9 | 98.16 | | |

## PRECISION

The study computed precision outcomes for mean measured concentration, standard deviation (SD), and percentage relative standard deviation (%RSD) pertaining to repeatability, intraday precision, and interday precision. The method developed was deemed acceptable and accurate based on the observation that the overall relative standard deviation (RSD) value was less than 2.

Table 5 : Repeatability table of paclitaxel

| Sample No | Theoretical Concentration (µg/ml) | Measured Concentration (µg/ml) | %Recovery | Mean Concentration (µg/ml) | SD | %RSD |
|---|---|---|---|---|---|---|
| 1 | 20 | 20.2 | 101 | 19.96 | 0.20 | 1.015 |
| 2 | 20 | 20.8 | 104 | | | |
| 3 | 20 | 19.8 | 99 | | | |
| 4 | 20 | 20.0 | 100 | | | |
| 5 | 20 | 19.4 | 97 | | | |
| 6 | 20 | 19.6 | 98 | | | |

Table 6: Interday Precision

| Theoretical Concentration (µg/ml) | Measured Concentration (µg/ml) Day1 | Measured Concentration (µg/ml) Day2 | Measured Concentration (µg/ml) Day3 | Mean %RSD |
|---|---|---|---|---|
| 20 | 20.80 | 19.30 | 20.64 | 1.06 |
| 20 | 19.60 | 20.10 | 20.41 | |
| 20 | 19.72 | 20.32 | 19.50 | |
| 20 | 19.32 | 19.78 | 20.80 | |
| 20 | 20.63 | 19.92 | 19.24 | |
| 20 | 20.42 | 20.11 | 19.82 | |
| Mean | 20.081 | 19.92 | 20.06 | |
| SD | 0.24 | 0.145 | 0.26 | |
| %RSD | 1.244 | 0.728 | 1.30 | |

Table 7 : Intraday Precision

| Theoretical Concentration (µg/ml) | Measured Concentration (µg/ml) 9:00 am | Measured Concentration (µg/ml) 11:00 am | Measured Concentration (µg/ml) 1:00 pm | Measured Concentration (µg/ml) 3:00 pm | Mean %RSD |
|---|---|---|---|---|---|
| 20 | 19.96 | 19.99 | 20.42 | 21.02 | 1.3 |
| 20 | 18.96 | 19.78 | 20.21 | 19.45 | |
| 20 | 19.80 | 19.63 | 19.87 | 18.89 | |
| 20 | 20.32 | 20.64 | 20.01 | 20.83 | |
| 20 | 20.24 | 21.40 | 20.80 | 20.11 | |
| 20 | 21.42 | 20.84 | 19.89 | 19.92 | |
| Mean | 20.116 | 20.38 | 20.2 | 20.03 | |
| SD | 0.32 | 0.28 | 0.14 | 0.33 | |
| %RSD | 1.62 | 1.38 | 0.72 | 1.64 | |

**ROBUSTNESS**

The percentage relative standard deviation (RSD) values were determined to be less than 2, indicating that the suggested approach exhibits robustness.

Table 8: Robustness table of paclitaxel

| Parameter | Changes | Theoretical Concentration (µg/ml) | Measured Concentration (µg/ml) | %Recovery | Mean, SD& %RSD |
|---|---|---|---|---|---|
| Flow Rate | 1.2 ml/min | 50 | 49.62 | 99.24 | Mean=49.86 |
| | 1.3 ml/min | 50 | 49.84 | 99.68 | SD= 0.144 |
| | 1.4 ml/min | 50 | 50.12 | 100.24 | %RSD=0.291 |
| Acetonitrile Variation | 43% | 50 | 49.28 | 99.56 | Mean=49.71 |
| | 45% | 50 | 49.87 | 99.74 | SD= 0.217 |
| | 48% | 50 | 49.98 | 99.96 | %RSD=0.437 |
| Methanol Variation | 43% | 50 | 49.58 | 99.16 | Mean=49.96 |
| | 45% | 50 | 50.22 | 100.44 | SD= 0.194 |
| | 48% | 50 | 50.08 | 100.16 | %RSD=0.388 |

**LOD and LOD**

The Limit of Detection (LOD) and Limit of Quantification (LOQ) are two important parameters used in analytical chemistry to determine the lowest concentration of an analyte that can be reliably detected and quantified, respectively. The limit of detection (LOD) and limit of Quantification (LOQ) for the High Performance Liquid Chromatography (HPLC) method proposed were determined to be 1.57µg/ml and 4.76µg/ml, respectively.

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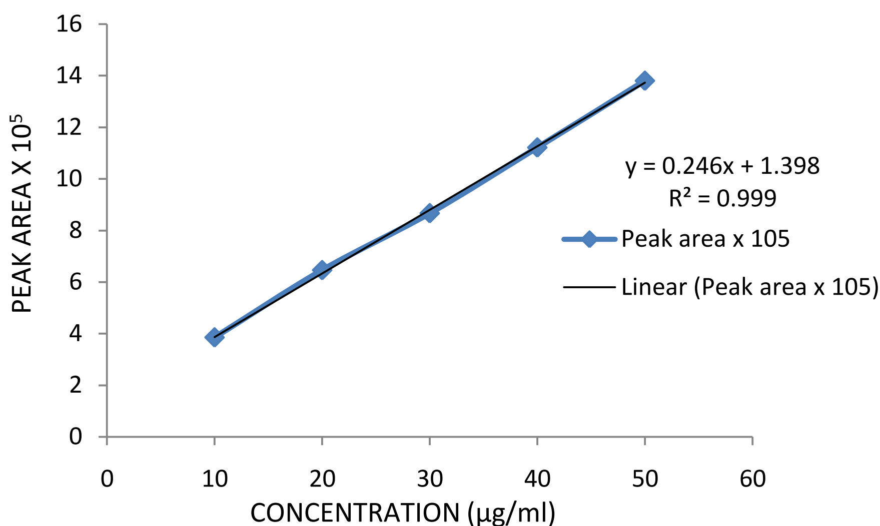

Figure 4: Calibration curve of paclitaxel (LOD and LOQ)

Table 9 : Regression statistics for LOD and LOQ

| Regression Statistics | |
|---|---|
| R Square | 0.999323 |
| Adjusted R Square | 0.9990989 |
| Standard Error | 0.117246 |
| Observations | 5 |

**LOQ :** $\frac{10\sigma}{\text{Slope}} = \frac{10 \text{ X } 0.117246}{0.246} = 4.76\mu g/ml.$

**LOD** : $\frac{3.3\ \sigma}{\text{Slope}} = \frac{3.3 \text{ X } 0.117246}{0.246} = 1.57\mu g/ml$

Table 10: Summary of method validation parameters of the proposed HPLC method

| PARAMETER | RESULT |
|---|---|
| Correlation Coefficient | 0.999 |
| Regression equation | y = 0.254x + 1.21 |
| Slope | 0.254 |
| Intercept | 1.21 |
| Accuracy | 98.58% - 99.33% |
| Precision | 1.01 % ( Repeatability)<br>1.06% (Interday)<br>1.3 % (Intraday) |
| LOD | 1.57µg/ml |
| LOQ | 4.76µg/ml |
| Robustness | 0.36 % |

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## V.CONCLUSION

In conclusion, the developed reverse phase high-performance liquid chromatography (RP-HPLC) method for the determination of paclitaxel has proven to be simple, accurate, sensitive, and reliable. The chromatographic conditions, including the use of acetonitrile: methanol (60:40) as the mobile phase, a flow rate of 1.5 ml/min, a wavelength of 227nm, an injection volume of 20µl, and a Symmetry C18 column, have been optimized for efficient separation and quantification of paclitaxel.

The validation of the method, conducted in accordance with the International Conference on Harmonization (ICH) guidelines, encompassed essential parameters such as linearity, accuracy, precision, robustness, limit of detection (LOD), and limit of quantification (LOQ). The correlation coefficient ($r^2$) for linearity was found to be 0.999, indicating an excellent relationship between the concentration of paclitaxel and peak area. The accuracy of the method was confirmed through recovery studies, with percentage recoveries falling within the acceptable range of 98.58% to 99.33%. Precision, evaluated through repeatability, interday, and intraday studies, demonstrated low percentage relative standard deviation (%RSD) values, confirming the method's reliability and consistency .The robustness of the method was assessed by introducing minor variations in parameters such as flow rate, acetonitrile concentration, and methanol concentration. The method exhibited stability and robustness, with %RSD values less than 2, indicating its ability to withstand minor changes without significantly affecting the results. The limits of detection (LOD) and quantification (LOQ) were determined to be 1.57µg/ml and 4.76µg/ml, respectively, highlighting the sensitivity of the developed method.

In summary, the proposed RP-HPLC method provides a valuable tool for the accurate determination of paclitaxel in pharmaceutical formulations and biological matrices. Its simplicity, precision, accuracy, and robustness make it suitable for routine analysis in the context of clinical research. The comprehensive validation results support the reliability and suitability of the method for its intended purpose in the pharmaceutical and analytical fields.

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