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Development and Validation of an RP-HPLC Analytical Method for Oxycodone in Pharmaceutical Dosage Forms

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ABSTRACT

This research focuses on development and validation of HPLC analysis of oxycodone in pharmaceutical dosage form. A mixture of methanol:acetonitrile:water (40:40:20% v/v) at pH 4.0 was identified as the optimal choice The established technique has been validated in accordance with ICH accepted standards. Linearity and range assessments established a linear range of 50–150 µg/ml with a high correlation coefficient of 0.997. Accuracy was confirmed through recovery studies, with recoveries ranging from 97.04% to 100.83% for oxycodone. Precision was evaluated through repeatability, intra-day, and inter-day precision studies, demonstrating low relative standard deviation (RSD) values Detection sensitivity was determined by the limits of detection (LOD) and quantification (LOQ), which were found to be 0.99 µg/ml and 3.01 µg/ml, respectively. The stability of standard and sample solutions was examined at room temperature for 48 hours, with a relative standard deviation below 2.0%. The determination of oxycodone in pharmaceutical dosage forms was made possible by the present validated technique, which has exceptional specificity, linearity, precision, sensitivity, and reliability.

Keywords: Oxycodone, validation, HPLC, precision, LOD & LOQ, stability.

1. INTRODUCTION

Due to unpredictability and changing circumstances, the rising number of medications in the marketplace, including novel constituents and revised variations may result in a delay in their inclusion in pharmacopoeias. The process of developing analytical techniques for such pharmaceuticals must be coordinated with the stages of drug development.[1,2]

In the central nervous system (CNS), oxycodone, a more effective and addictive narcotic analgesic than codeine, mainly targets mu-type receptors, causing a drop in cAMP levels that prevents the release of several neurotransmitters. Oxycodone also affects potassium and calcium channels, which lowers neural excitability.[3] A 1:2 equivalency to morphine is shown by oxycodone. In controlled-release formulations, it acts around an hour after ingestion and lasts for 1-2 hours. Within 24 hours, steady plasma levels are attained, and the plasma half-life is 3-5 hours. The range of oral bioavailability is 65% to 89%. Because oxycodone's metabolism is more consistent than morphine's, titration is simpler and less likely to result in unpleasant side effects including nausea, delusions, and discomfort. Effective pain treatment is achieved with its widespread use, which extends to postoperative care and cancer patients.[4,5]

It became clear from the literature research that there is no compendia approach that has been devised for evaluating oxycodone both in bulk and prescription dose forms.[5-10] The primary goal of the present research was to establish an effective, inexpensive, and validated procedure for selecting

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the solvents for HPLC technique development after determining the absorption maxima of oxycodone in the UV-Visible region using different solvents, optimize the mobile phase and flow rates for proper resolution and retention times, evaluate oxycodone in bulk drug substance, and finished products. The ICH guidelines were strictly followed while validating the chromatographic settings.

2. MATERIAL & METHODS

2.1 Chemicals and Instruments

The equipment utilized included a Nicolet Evolution 100 UV-Visible double beam spectrophotometer, an HPLC system (Shimadzu CHT-2010) with LC Solutions software, and a Mettler Toledo electronic analytical balance for weighing. A Hamilton syringe, Citizen digital sonicator, and Global Digital pH meter were employed. All reagents and solutions used in the study met HPLC analytical grade standards. The oxycodone in its API form was provided as a gift sample by Chandra Labs, Hyderabad, India.

2.2 Chromatographic Parameters

The analysis utilized an Inertsil ODS-3V HPLC column measuring 150 x 4.6 mm with an internal diameter and 5-micron particle size. A UV detector was configured at 230 nm. The mobile phase consisted of a mixture of methanol, acetonitrile, and water in a 40:40:20% v/v ratio at pH 4.0, flowing at a rate of 1.0 ml/min. The retention time for oxycodone was determined to be 2.136 minutes, and each injection comprised 10 µl.

2.3 Preparation of Mobile Phase

Prepared a combination of methanol, acetonitrile, and water in the ratio of 40:40:20% v/v, mixed and sonicated.

2.4 Preparation of Standard Solution

Accurately weighed 10 mg of oxycodone and dissolved it in a 25 ml volumetric flask with 25 ml of the mobile phase, adjusting the volume with the mobile phase. To obtain a 20 µg/ml oxycodone solution, 0.5 ml of the stock solution was diluted to 10 ml using the mobile phase.

2.5 Preparation of Standard Solution

Accurately weighed 10 mg of oxycodone (obtained from oxycodone market tablets) into a 25 ml volumetric flask, dissolved it in 25 ml of the mobile phase, and adjusted the volume with the mobile phase. Subsequently, a 20 µg/ml oxycodone solution was prepared by diluting 0.5 ml of the stock solution to 10 ml using the mobile phase.

2.6 Preparation of Standard Solution

The developed method was validated for various parameters including linearity and range, precision, accuracy, LOQ and LOD, specificity, robustness, and stability.[11-13]

2.6.1 Linearity and Range

To assess the linearity of the analytical method, five working standard solutions were separately injected into the HPLC system in triplicate at various concentration levels ranging from 0.15 to 2 mg/mL. A calibration curve was then generated, plotting the concentration of oxycodone on the x-axis and the average peak area on the y-axis. Linear regression analysis was employed to establish the regression equation and determine the correlation coefficient.

2.6.2 Accuracy and Precision

Inter-day precision was assessed by evaluating three quality control samples on 3 separate days, while intra-day precision was determined by analysing the same samples three times on a single day, all at three distinct concentration levels (50, 100, 150 mg). The relative standard deviation (RSD) values were calculated for both intra-day and inter-day analyses, with the acceptance range set at no more than 2%, to calculate the precision of the proposed technique. Practical accuracy was measured with the % deviation between the observed concentration of the QC sample and the expected concentration.

2.6.3 LOQ and LOD

Limits of detection (LOD) and quantification (LOQ) were determined based on signal-to-noise ratios of 3:1 and 10:1, respectively in accordance with the equation recommended by ICH guidelines.

$$\text{LOD} = \frac{3.3\sigma}{S} \qquad \text{LOQ} = \frac{10\sigma}{S}$$

2.6.4 Specificity

By injecting blank samples, the method's specificity was examined to show that there was no interference with the oxycodone elution in standard samples or pharmaceutical formulations.

2.6.5 Stability

Stability of the samples was tested by duplicated analysis (n = 3) to assess sample stability during the

analysis for 48 h at room temperature.

3. RESULTS

3.1 Optimization of Mobile Phase

During the initial method development stage, various combinations of methanol, acetonitrile, and water were experimented with as potential mobile phases. Ultimately, a system comprising a mixture of methanol:acetonitrile:water in a 40:40:20% v/v ratio (at pH 4.0) was identified as satisfactory, yielding a well-defined peak for oxycodone. The retention time for oxycodone was 2.92 minutes. The achieved resolution exceeded 1.0, indicating successful separation of the compound. Figure 1 illustrates the blank and standard chromatograms for oxycodone optimization.

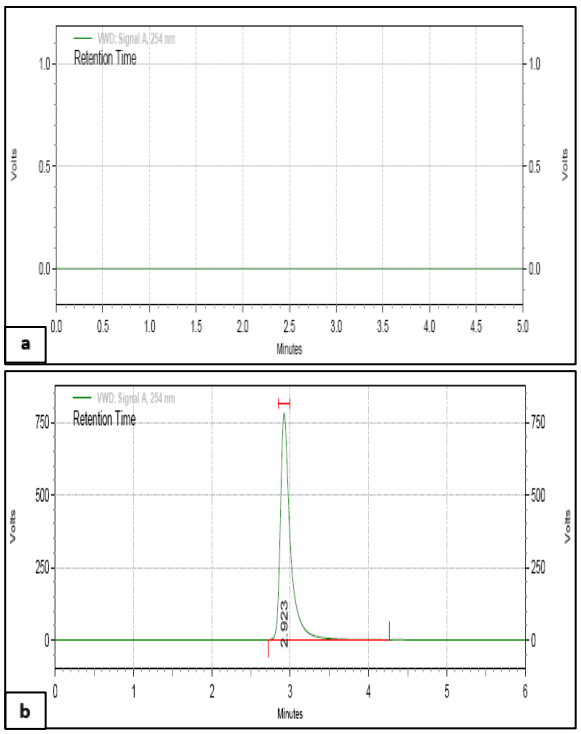

Figure 1: Optimization of oxycodone
Chromatogram of a) Blank solution b) Standard solution

3.2 System Suitability

System suitability was assessed to verify the adequacy and consistency of the chromatographic system for the analysis. This evaluation was conducted prior to sample analysis, involving duplicate injections of the standard solution containing 20 µg/mL of oxycodone. The acceptance criterion was set at a USP tailing factor of no more than 2.0. All critical parameters examined adhered to the predefined acceptance criteria.

3.3 Linearity and Range

The calibration curve for oxycodone shown linearity within the range of 50–150 µg/ml with a high correlation coefficient of 0.997. A detailed presentation of the regression analysis of the calibration curves is shown in Figure 2 and Table 1.

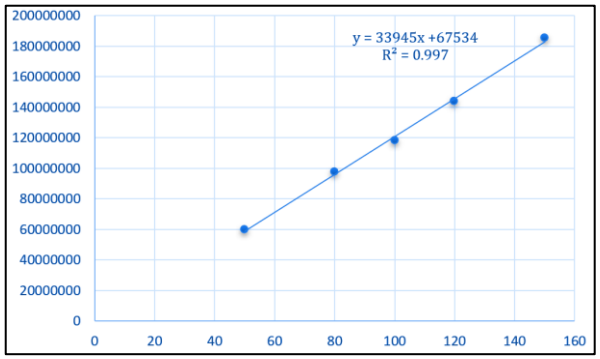

Figure 2: Linearity graph of oxycodone

S. No	Parameters	Oxycodone
1	Range	50-150 µg/ml
2	Correlation Coefficient	0.997
3	Slope	33945

Table 1: Linearity and precision of oxycodone

3.4 Accuracy

Method accuracy was assessed through recovery studies, which involved adding reference standards of the drugs to the formulation at levels of 50%, 100%, and 150%. The recoveries obtained for oxycodone ranged from 97.04% to 100.83%. These recovery experiments were conducted three times, and the results, including percentage recovery and percentage mean recovery for the drug, are presented in Table 2.

Level of % Recovery	Mean % Recovery	% RSD
50	97.04	0.098
100	99.64	0.233
150	99.11	1.410

Table 2: Recovery of oxycodone

3.5 Precision

Method precision was ascertained through repeatability with RSD values of 0.070% for oxycodone in the present study, as shown in Figure 3. Intra-day and inter-day precision studies were conducted, and the observed low RSD values serve as evidence of the method's precision.

3.6 Limit of Detection (LOD) and Limit of Quantification (LOQ)

The detection limits for oxycodone were 0.99 µg/ml while quantitation limits were 3.01 µg/ml respectively. The data demonstrates that the method allows for the accurate and precise determination of even nanogram quantities of both drugs.

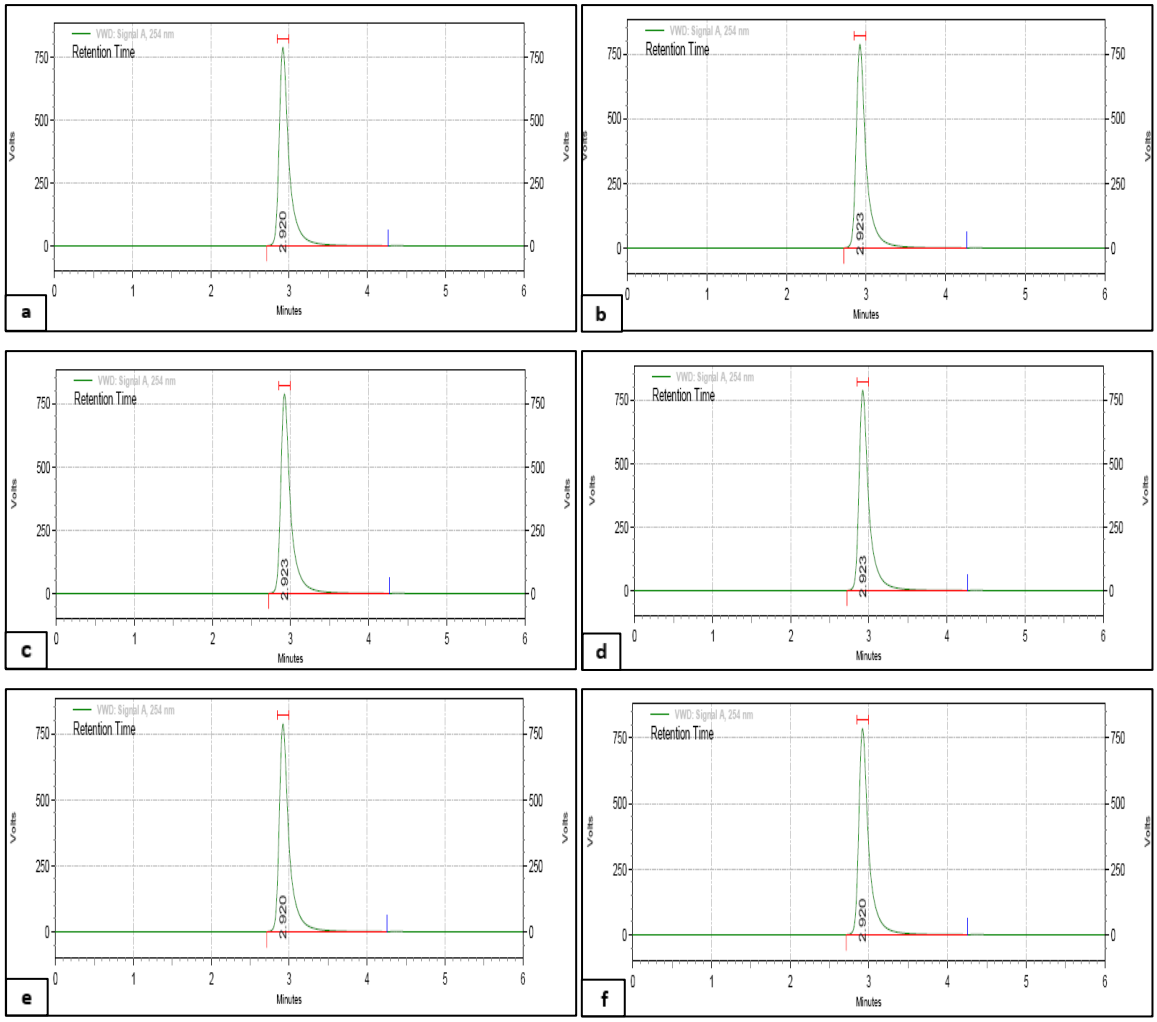

Figure 3: Precision of oxycodone

Chromatogram of a, b & c represent inter-day precision studies; d, e & f represent intra-day precision studies.

3.7 Specificity

The specificity assessment was conducted to examine potential interference from the excipients employed in the formulations. In accordance with the test technique, analysis was done on a placebo in triplicate, about comparable to the weight of the placebo in test preparation. Chromatograms of placebo and blank solutions exhibited no peaks at oxycodone retention periods (Figure 4).

3.8 Robustness

To assess the robustness of the developed method, deliberate alterations were made to the experimental conditions, and the retention time of oxycodone was recorded in Table 3. The variables evaluated in the study were column temperature (± 5°C) and flow rate (± 0.2 mL/min). In all the deliberate varied chromatographic conditions, all analytes were adequately resolved, and the elution

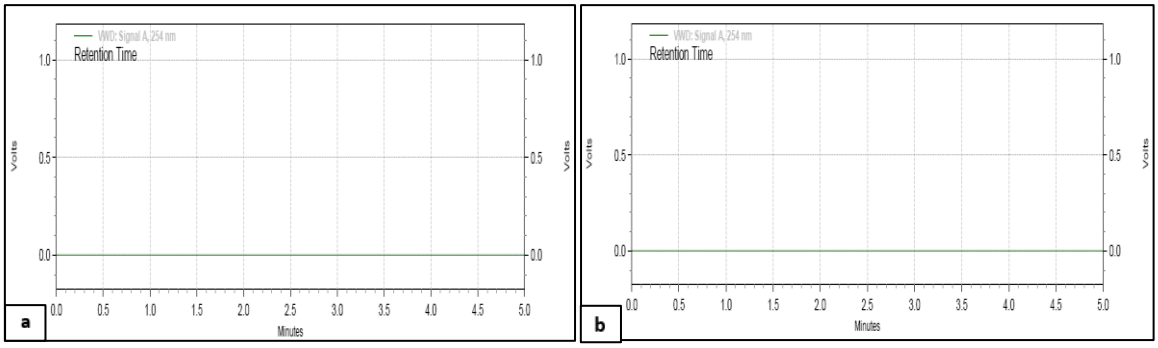

Figure 4: Specificity of oxycodone

Chromatograms of a) Placebo solution b) Blank solution

order remained unchanged.

Chromatographic Changes		Rt (min)	Tailing Factor	%RSD
Flow Rate (mL/min)	0.4	4.389	1.69	0.2
	0.6	1.947	1.66	0.08
Temperature (°C)	25	2.937	1.76	0.03
	35	2.91	1.78	0.1

Rt= Retention time; %RDS: relative standard deviation
Table 3: Robustness of the developed HPLC method

3.9 Stability

The stability of the standard and sample solutions of oxycodone was assessed at room temperature over a 48-hour period. The relative standard deviation was found to be below 2.0%. It showed that both standard and sample solution were stable up to 48 h at room temperature.

4. DISCUSSION

The present research described the optimization of the mobile phase for the analysis of oxycodone using high-performance liquid chromatography (HPLC). Different mixtures of methanol, acetonitrile, and water were used to find an optimal mobile phase. Ultimately, a mixture of methanol:acetonitrile:water (40:40:20% v/v) at pH 4.0 was identified as the most suitable, yielding a well-resolved peak for oxycodone with a retention time of 2.92 minutes and a resolution exceeding 1.0, indicating effective compound separation. The study then delves into the validation of the proposed method, covering various parameters to ensure its reliability and accuracy. System suitability was assessed, with acceptance criteria met for the tailing factor and area similarity ratio.

Linearity and range were established for oxycodone, with a linear range of 50–150 µg/ml and a high correlation coefficient of 0.997. Accuracy is determined by calculating the percentage of recovery through the assay of the known additional amount of analyte in the sample or by assessing the difference between the mean and the recognized true value. This assessment involves the consideration of confidence intervals.[11]

According to the ICH guidelines, accuracy should be evaluated across a minimum of three concentration values that span the required range. In the present study, accuracy was confirmed through recovery studies, which yielded recoveries ranging from 97.04% to 100.83% for oxycodone. The findings demonstrate a strong association between peak area and concentration of oxycodone.

When the procedure was repeatedly applied to several samplings of a homogenous sample, the accuracy of an analytical method is measured by the degree of concordance between single test findings. It is possible to derive statistically precise estimations of the standard deviation by assaying an adequate number of aliquots of a homogeneous sample, which is how the precision of a method of analysis is assessed.[11] In the present study, precision was evaluated through repeatability, intra-day, and inter-day precision studies, with low relative standard deviation (RSD) values, indicating the method's precision. The limits of detection (LOD) and quantification (LOQ), which were determined to be 0.99 µg/ml and 3.01 µg/ml, respectively, established accurate and precise detection of minute quantities of the drug.

Specificity was assessed to ensure that excipients in formulations did not interfere with the analysis, demonstrating that there were no peaks at oxycodone retention times in placebo and blank solutions. Robustness was also investigated by intentionally altering experimental conditions, and the results indicated that the method remained robust even with variations in column temperature and flow rate.

Finally, the stability of standard and sample solutions was evaluated at room temperature for 48 hours, with the relative standard deviation found to be below 2.0%. This stability data indicates that both the standard and sample solutions of oxycodone can be reliably stored and used at room temperature for up to 48 hours.

5. CONCLUSION

The quantitative measurement of oxycodone in pharmaceutical dosage forms was formulated and established using a quick and effective reverse-phase HPLC approach. During validation, the method was discovered to be exact, precise, accurate, linear, robust, and stable. Results from the validation of the procedure were satisfactory. The technique may be applied to regular manufacturing sample analysis and for practical applications in pharmaceutical and analytical laboratories.

Conflict of Interest: The authors did not declare any conflict of interests.

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