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HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF PROPRANOLOL AND FLUNARIZINE IN COMBINED DOSAGE FORM

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Abstract

This work presents the development and validation of a high-performance liquid chromatography (HPLC) method for the simultaneous estimation of Propranolol (PNL) and Flunarizine (FNZ) in pharmaceutical formulations. The method was subjected to a comprehensive validation process, including linearity, recovery studies, precision assessment, sensitivity determination, and assay of tablet formulations. The results demonstrated the method's accuracy, precision, and sensitivity, making it a robust tool for routine analysis in pharmaceutical laboratories. The successful application of the method to assess the assay of tablet formulations further underlines its practical utility in pharmaceutical quality control.

Keywords: HPLC, Propranolol, Flunarizine, Method Development, Method Validation, Pharmaceutical Formulations, Quality Control.

Introduction

High-Performance Liquid Chromatography (HPLC) stands as a cornerstone in pharmaceutical analysis, offering unparalleled precision, sensitivity, and versatility. It has become an indispensable tool for the quantification of pharmaceutical compounds in complex matrices. The development and validation of HPLC methods play a pivotal

role in ensuring the reliability and accuracy of analytical results, particularly in the pharmaceutical industry where stringent quality standards are paramount [1].

HPLC offers several advantages, including rapid separation, high resolution, and the ability to handle a wide range of analytes. As pharmaceutical formulations become more complex, the demand for robust and selective HPLC methods intensifies [2]. The method development process involves optimizing parameters such as mobile phase composition, column selection, and detection wavelength to achieve efficient separation and quantification [3].

Combination drug therapies are frequently employed in the treatment of various medical conditions to achieve synergistic effects, enhance therapeutic outcomes, and improve patient compliance [4]. Propranolol, a non-selective beta-blocker, and flunarizine, a calcium channel blocker, are often co-administered to manage conditions such as migraine headaches and cardiovascular disorders. Reliable and accurate quantification of both drugs in a combined dosage form is crucial for ensuring proper dosing and therapeutic efficacy [5].

High-Performance Liquid Chromatography (HPLC) is a widely utilized analytical technique known for its precision and sensitivity in pharmaceutical analysis [6]. The development and validation of an HPLC method for the simultaneous estimation of propranolol and flunarizine in a combined dosage form are essential for quality control and regulatory compliance.

Material and Methods

Instrument

Liquid chromatographic system from Waters model no 784 comprising of manual injector, water 515 binary pump for constant flow and constant pressure delivery and UV-Visible detector connected to software Data Ace for controlling the instrumentation as well as processing the generated data.

Methods

Selection of Mobile Phase

Initially to estimate Propranolol and Flunarizine in fix dosage form number of mobile phase in different ratio were tried. Taking into consideration the system suitability parameter like RT, Tailing factor, No. of theoretical plates and HETP, the mobile phase found to be most suitable for analysis was Acetonitrile: Methanol in the ratio of 50:50v/v. The mobile phase was filtered through 0.45µ filter paper to remove particulate matter and then degassed by sonication. Flow rate employed for analysis was 1.0 ml/min.

Preparation of Stock Solution:

Accurately weighed 10 mg API of PNL and FNZ was transferred into 10 ml volumetric flask separately and added 5ml of methanol as diluents, sonicated for 20 minutes and volume was made up to 10ml with methanol to get concentration of solution 1000µg/ml (Stock-A)

Preparation of Sub Stock Solution:

5 ml of solution was taken from stock-A of both the drug and transferred into 50ml volumetric flask separately and diluted up to 50 ml with diluent (methanol) to give concentration of 100µg/ml of PNL and FNZ respectively (Stock-B).

Preparation of Different Solution

1ml, 2ml, 3ml, 4ml and 5ml of stock-B were taken separately in 10 ml volumetric flask and volume was made up to 10ml with (methanol). This gives the solutions of 10µg/ml, 20µg/ml, 30µg/ml, 40µg/ml and 50µg/ml, for PNL. In same manner 5µg/ml, 10µg/ml, 15µg/ml, 20µg/ml and 25µg/ml of FNZ also prepared [59].

Linearity and Calibration Graph

To establish the linearity of analytical method, a series of dilution ranging from 10-50 µg/ml for PNL and 5-25µg/ml for FNZ were prepared. All the solution were filtered through 0.45µm membrane filter and injected, chromatograms were recorded and it was repeat for five times. A calibration graph was plotted between the mean peak area and respective concentration and regression equation was derived [60].

System Suitability Parameters

Separation variables were set and mobile phase was allowed to saturate the column at 1.00 ml/min. After complete saturation of column, six replicates of working standard of PNL 10μg/ml for PNL and 10μg/ml FNZ was injected separately. Peak report and column performance report were recorded for all chromatogram.

Validation of developed Method

The method was validated for the parameters reported below [7].

A. Linearity

Linearity of analytical procedure is its ability (within a given range) to obtain test which are directly proportional to area of analyte in the sample. The calibration plot was contracted after analysis of five different concentrations (from 10 to 50μg/ ml for PNL) and (5 to 25μg/ ml for (FNZ) and areas for each concentration were recorded three times and mean area was calculated. The response ratio (response factor) was found by dividing the AUC with respective concentration.

Specificity

Specificity of the method was carried out to assess unequivocally the analyte presence of the components that might be expected to be present such as impurities, degradation products and matrix components.

Accuracy

Recovery studies were performed to calculate the accuracy of developed method to preanalysed sample solution, a definite concentration of standard drug (80%, 100%, and 120%) was added and then its recovery was analyzed.

Precision

The stock solution was prepared. The precision are established in three differences:

Repeatability

The repeatability was performed for five replicate at five concentrations in linearity range 1, 2, 3, 4 and 5μg/ml for PNL and 5, 10, 15, 20 and 25μg/ml for FNZ indicates the precision under the same operating condition over short interval time.

Day To Day Precision

Intermediate precision was also performed within laboratory variation on different days and different analyst in five replicate at five concentrations. Results of day to day intermediate precision for PNL and FNZ.

Robustness

As per ICH norms, small but deliberate variations in concentration of the mobile phase were made to check the method's capacity to remain unaffected. The ratio of mobile phase was change from, Acetonitrile: Methanol (50:50 % v/v) to (45:55 % v/v).

Detection Limit and Quantitation Limit

The LOD and LOQ of developed method were calculated based on the standard deviation of response and slope of the linearity curve.

Analysis of both the drug in Tablet Sample

Twenty tablets were accurately weighed and their mean weight was determined. The tablets were grinded to fine powder, an accurately weighed quantity of powder equivalent to 20 mg of PNL was transferred to 10 ml volumetric flask containing methanol. The solution was sonicated for 25 min and the final volume was made with mobile phase. The mixture was then filtered through a 0.45 µm filter. The stock solution was further diluted sufficiently with methanol to get sample solution of drug concentration of 20µg/mL PNL and 5µg/mL FNZ respectively. The amounts of PNL and FNZ in tablets formulation were calculated by extrapolating the value of area from the calibration curve. Analysis procedure was repeated six times with formulation.

Results and Discussion

The linearity of the HPLC method for the simultaneous determination of Propranolol (PNL) and Flunarizine (FNZ) was evaluated over the concentration ranges of 10-50 µg/ml for PNL and 5-25 µg/ml for FNZ. The correlation coefficients (r2) obtained for both analytes were indicative of excellent linearity. The average slope (m) and intercept (c) values further reinforced the linearity of the calibration curves, ensuring a reliable quantitative analysis.

Recovery studies were conducted on marketed formulations at three different levels (80%, 100%, and 120%). The percentage recovery values, along with their standard deviations, demonstrated the accuracy and precision of the developed HPLC method. The results indicated that the method is suitable for the quantification of PNL and FNZ in complex matrices, such as pharmaceutical formulations, with satisfactory recovery rates at different concentration levels.

The method's precision was assessed through repeatability, day-to-day, and analyst-to-analyst variations. The low percentage relative standard deviations (%RSD) obtained for these parameters indicated the method's repeatability and robustness. The values within the acceptable range demonstrated the reliability of the method for routine analysis in a laboratory setting.

The limits of detection (LOD) and quantification (LOQ) were determined to assess the method's sensitivity. The low LOD and LOQ values indicated the method's capability to detect and quantify PNL and FNZ at very low concentrations. This sensitivity is crucial for accurately quantifying analytes in pharmaceutical formulations, where low levels of active ingredients may be present.

The developed HPLC method was applied to assess the assay of tablet formulations containing PNL and FNZ. The percentage of the label claim found for both analytes demonstrated the accuracy of the method in determining the actual drug content in the formulations. The low % RSD values indicated the precision and reproducibility of the assay results.

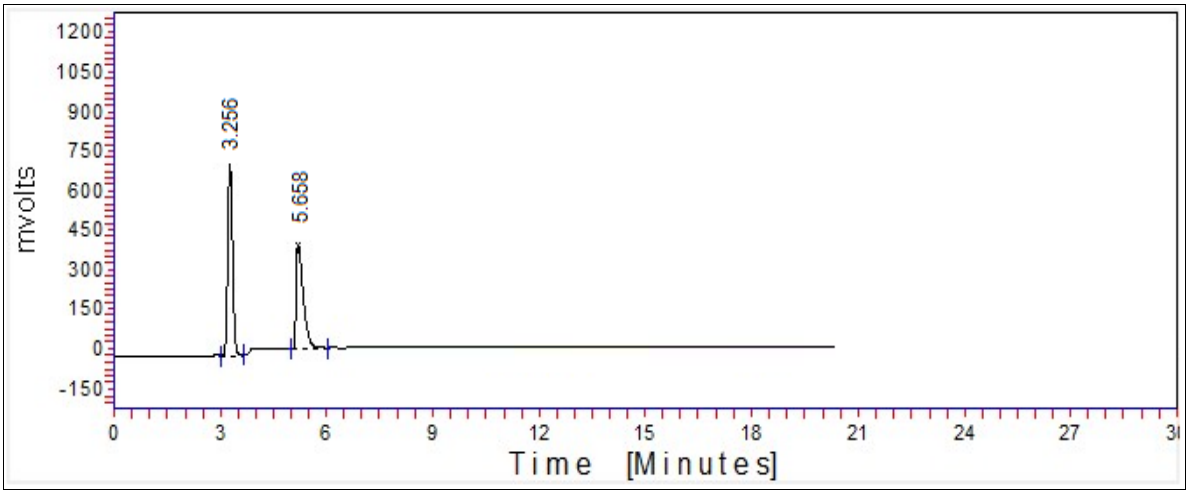

Figure 1: Chromatogram of Both the drug

Table 1: Results of Linearity of Propranolol (PNL) and Flunarizine (FNZ)

S. No.	Parameter	PNL	FNZ
1	Linearity	10-50µg/ml	5-25µg/ml
2	Correlation Coefficient (r^2)*	0.990	0.999
3	Slope (m)*	50.01	50.07
4	Intercept (c)*	62.92	7.048

*Average of five determination

Table 2: Results of Recovery Studies on Marketed Formulations

Recovery Level %	% Recovery (Mean±SD)*	
	PNL	FNZ
80	99.01±0.255	97.66±1.338
100	98.72±0.842	98.50±0.726
120	99.06±0.294	98.42±0.869

Table 3: Results of validation (%R.S.D.)

PARAMETER		(Mean±SD)	
		PNL	FNZ
Precision (%R.S.D.)*	Repeatability	99.312±0.110	98.341±0.111
	Day to Day	99.033±0.117	98.405±0.079
	Analyst to Analyst	99.201±0.083	99.523±0.047

	Robustness	99.093±0.101	90.376±1.047

*Average of five determination

Table 4: LOD and LOQ of PNL and FNZ

Name	LOD (µg/ml)	LOQ (µg/ml)
PNL	0.45	1.25
FNZ	0.30	0.90

Table 5: Result of assay of tablet formulation

	PNL*	FNZ*
Label Claim (mg)	**40mg**	**10mg**
% Found (mg)	39.82	8.92
% Assay	99.55	99.20
% RSD	0.125	0.162

*Average of three determination

Conclusion

The successfully validated HPLC method provides a reliable and efficient means for the simultaneous determination of Propranolol and Flunarizine in pharmaceutical formulations. The findings contribute to the analytical methods available for quality control in the pharmaceutical industry, ensuring the accurate assessment of drug content in complex matrices. The robustness and applicability of the method underscore its potential for routine use in pharmaceutical laboratories, supporting the broader goal of ensuring the safety and efficacy of pharmaceutical products in the market.

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