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

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ABSTRACT

Aim: The aim of this study is to develop and validate a high-performance liquid chromatography (HPLC) method for the simultaneous estimation of Propranolol and Flunarizine in a combined dosage form. The developed method aims to provide a reliable, accurate, and efficient means of quantifying both drugs in the pharmaceutical formulation. **Method Development:** To optimize chromatographic conditions for the separation of Propranolol and Flunarizine. To select a suitable stationary phase, mobile phase composition, and gradient elution program for efficient separation. To achieve baseline resolution and adequate peak shapes for both compounds. **Method Validation:** To validate the developed HPLC method according to regulatory guidelines. To evaluate the specificity of the method by ensuring that excipients or other potential impurities do not interfere with the detection of Propranolol and Flunarizine. To establish linearity over a range of concentrations for both Propranolol and Flunarizine. To determine the precision of the method, including repeatability, intermediate precision (day-to-day and analyst-to-analyst variations), and reproducibility. To assess the accuracy of the method by conducting recovery studies on the combined dosage form. To establish the limits of detection (LOD) and quantification (LOQ) for both Propranolol and Flunarizine. **Conclusion:** Modern medicines for human use are required to comply with specific standards and regulation set forth by the concerned authorities. The efficacy and safety of medicinal products can only be assured by analytical monitoring of its quality. Pharmaceutical analysis is an art and science of determining the concentration of drug constituents present in marketed formulation. It is considered as an application of procedures necessary to determine and estimate the identity, strength, quality and purity of drug. Therefore, the quality control laboratory is considered as the backbone of the pharma industries with ever- increasing need for the development of analytical techniques for drug formulation.

KEYWORDS: HPLC, Propranolol, Flunarizine, Pharmaceutical formulation.

1. INTRODUCTION

Drug is broadly defined as “any chemical agent that affects living processes”, As drug involves life, its quality becomes most important. So drug should be available in a form in which quality including bioavailability, proper plasma concentration, desired duration of action, rapid onset, accurate dose, safety, efficacy and stability on storage of product, can be assured. The WHO has defined essential drugs as, those that satisfy the healthcare needs of majority of the population; they should be available at all times in accurate amount end in appropriate dosage form. So quality of pharmaceuticals has to be monitored from the very beginning i.e. from raw material to the end, i.e. finished product. Quality control is a concept that strives to produce an ideal product by series of measures, designed to prevent and eliminate errors at different stages in production that is why at various stages, analysis of product is done. Chemical analysis can be defined as the resolution of a chemical compound into its proximate parts; the determining its elements or the foreign substances it may contain. The drug products are required by law to confirm a minimum standard of quality. With the rapid development of pharmaceuticals and higher challenges of quality, the volume of analytical work is increasing day by day. This forces the development of analytical methods that are rapid, accurate, precise and reproducible. Analytical chemistry plays vital role in development of science, which involves separation, identification and determination of the relative amounts of components in a sample of matter.[1-2]

1.1 Types of chemical analysis

1.1.1 Qualitative Method

When a completely unknown sample is taken for analysis, presence of one or more component in the sample and prediction of structural features of the compound, are done by qualitative method.[4-5]

1.1.2 Quantitative Method

This method deals with the determination of quantity i.e. content of each component or specified component of the sample, whose chemical behavior is known.[6]

1.2 Introduction to chromatography

Today, chromatographic techniques have little to do with the separation of color (the technique names evolved from the earliest work of separating dyes or plant pigments on paper), but do involve the separation of compounds in a sample mixture. A number of types of separation methods have developed over the years to accommodate the various physical and chemical states of sample mixtures one may be interested in separating and analyzing. The feature that distinguishes chromatography from most other physical and chemical methods of separation is that, two mutually immiscible phases brought into contact; one phase is stationary and other mobile. The mobile phase can be gas or a liquid, whereas the stationary phase can only be a liquid or a solid. When the separation involves predominantly a simple portioning between two immiscible liquid phases, one stationary and other mobile, the process is called liquid-liquid chromatography. When physical surface forces are mainly involved in the retentive ability of the stationary phase, the process is denoted as liquid solid chromatography. Liquid chromatography has been performed in a column or on an open bed.[9-12]

1.2.1 Choice of Chromatographic Method

The choice of method must be largely empirical as there is no way of predicting the best procedure for separation, except in some simple cases. It is usual to try simpler techniques such as Paper Chromatography or Thin Layer Chromatography first, as they can often provide a useful guide to the type of system for separation. The following list gives a rough guideline for the choice of method.[8,14]

Table 1.1: Choice of Method.

Substance of similar chemical type	Partition Chromatography
Substance of different chemical type	Adsorption Chromatography
Gases and volatile Substance	Gas Chromatography
Ionic and Inorganic Substance	Ion Exchange Chromatography, Paper or Thin Layer Chromatography
Ionic form non-ionic Substance	Ion Exchange or Gel Chromatography
Biological materials, compounds of high molecular weight	Gel Chromatography

1.3 High performance liquid chromatography

Early in the development of liquid chromatography, scientist realized that increase in column efficiency could be brought about by decreasing the partial size of packaging. The technology for producing and using pickings with particle diameter as smalls 3 to10µm was developed. This technology required sophisticated instruments operating at high pressure, which contrasted markedly with the simple glass columns of classic gravity-flow liquid chromatography. The name High Performance Liquid chromatography (HPLC) is employed to distinguish this newer procedure.[15]

1.4 HPLC instrumentation: An overview

The essential parts of the HPLC are the solvent reservoir tubing, pump, injection device, column, detector and recorder. Its simplicity, high specificity and wide range of sensitivity make it ideal for the analysis of many drugs in both dosage forms and biological fluids.

1.4.1 Mobile Phase Reservoir and Solvent Treatment System

A modern HPLC apparatus is equipped with one or more glass or stainless steel reservoir the composition of the reservoir should be inert to a variety of aqueous and non-aqueous mobile phase, each of which contains 500ml or more solvent. Stainless steel should be avoided for solvent containing halide ions and glass should be avoided, if the reservoir is to be pressurized. The reservoir often equipped with mean of removing dissolved gases usually O_2 and N_2 that interfere by forming bubbles in the columns systemty, viscosity, compressibility, refractive index, UV cut off, polarity, vapor pressure, flash point, degassing and purity of the solvents are considered in the selection of mobile phase.

Solubility play critical role in partition chromatography, it shows a degree of similarity of different molecule. Compound with similar solubility mix easily, with no thermodynamic energy requirement. Viscosity of the mobile phases increases, the efficiency of the system, as measured by the number of theoretical plates decreases, compressibility is the tendency of a solvent to be compressed in volume during the pumping cycle solvent with high degree of compressibility may show greater pulsations with reciprocating pumps, denser solvents are less compressible.

Than adequate, after a few additional trials, it may be tempting to accept a marginal separation, especially if no further improvement is observed.

1.5 Analytical method validation

The concept of validation was formally introduced in the USA, in early 1979, its scope, since then has got expanded to include a wide range of function related to the pharmaceutical development & manufacturing. Analytical data are used to screen potential drug candidates aid in the development of drug synthesis, formulation studies monitor the stability of bulk pharmaceuticals and formulated products, and test final products for release. The quality of analytical data is a key factor in the success of a drug development program. So the process of method development and validation has direct impact on the quality of pharmaceutical products.

1.5.1 General Concept of Validation

The general concept of validation is that, the valid method:-

- Provides useful analytical data in a specific situation
- Is suitable for its intended use
- Meets predetermined requirement (specifications) of the analytical problem.
- Has established level performance like accuracy, consistency, reliability etc.

1.5.2 Need of Method Validation

A method should be validated when it is necessary to verify that, the performance parameters are adequate for use for particular analytical problem, for example-

- When the established method needs improvement.
- Development of new method for particular problem.
- Established method used in a different laboratory or with different analysis or different instrumentation.
- To demonstrate the equivalence between two methods e.g. a new method and a standard method. Impossible to obtain samples of certain impurities and/or degradation products, it is considered acceptable to compare results obtained by an independent procedure. The response factor of the drug substance can be used. It should be clear how the individual or total impurities are to be determined e.g. weight/weight or area percent, in all cases with respect to the major analyte.

Recommended data: Accuracy should be assessed using a minimum of 9 determinations over a minimum of 3 concentration levels covering the specified range (e.g. 3 concentrations/3 replicates each of the total analytical procedure). Accuracy should be reported as percent recovery by the assay of known added amount of analyte in the sample or as the difference between the mean and the accepted true value together with the confidence intervals. Detection limit is determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be reliable detected.

Based on signal-to-noise: This approach can only be applied to analytical procedures, which exhibit baseline noise. Determination of the signal-to-noise ratio is performed by comparing measured signals from samples with known low concentrations of analyte with those of blank sample and establishing the minimum concentration at which the analyte an be reliable detected. A signal-to-noise ratio between 3 or 2:1 or generally considered acceptable for estimating the detection limit.

Based on the standard deviation of the response and slope

The detection limit (DL) may expressed as:

- $DL = \frac{3.3\ \square}{S}$

Where

$\square$ = the standard deviation of the response S = the slope of the calibration curve.

The slope S may be estimated from the calibration curve of the analyte. The estimate of $\square\square$may be carried out in a variety of ways, for example:

Based on the standard deviation of the blank: Measurement of the magnitude of analytical background response is performed by analyzing an appropriate number of blank sample and calculating the standard deviation of these responses.

Based on the calibration curve: A specific calibration curve should be studied using samples containing an analyte in the range of DL. The residual standard deviation of a regression line or the standard deviation of y-intercepts of regression lines may be used s the standard deviation.

Recommended data: The detection limit and the method used for determining the detection limit should be presented. If DL is determined based on visual evaluation or based on signal to nose ratio, the presentation of the relevant chromatograms is considered acceptable for justification. In cases where an estimated value for the detection limit is obtained by calculation or extrapolation, this estimate may subsequently be the independent analysis of a suitable number of sample known to be near or prepared at the detection limit.

2. EXPERIMENTAL WORK AND RESULTS

2.1 Identification and characterization of drugs

2.1.1 Physical characterization of drug

The drugs PNL and FNZ were physically characterized on the beginning of appearance, color and odor. All these parameter were recorded and compared with the literature.

2.1.2 Melting point determination

The melting point determined used for the strength of mind of melting point of PNL and FNZ by the open capillary methods. The melting point of drug was recorded and compared with literature values. The Melting point of PNL and FNZ was found 163- 164 ^{0}C and 250-252^{0}C respectively.

2.2 Proposed method

Method development and validation of Propranolol (PNL) and Flunarizine (FNZ) using RP-HPLC.

Theme

✓Determination of solubility
✓Selection of Mobile Phase
✓Selection of Flow rate
✓Wavelength selection for linearity study
✓Linearity range and calibration graph
✓Assay of marketed formulation
✓Validation of developed method

2.2.1 Solubility

Solubility of PNL and FNZ was determined at 25±1°C. Accurately weighed 10 mg PNL and FNZ was added in different 10 ml volumetric flask containing different solvent and placed at mechanical shaker for 8 hrs. After 8 hrs filter both solution were filtered through whatman filter paper No. 41. The filtrates were diluted suitably and analyzed spectrophotometrically against water.

Table 2.1: Solubility of drugs in different solvents.

Solvent	Solubility	
	Propranolol	**Flunarizine**
Water	Sparingly Soluble	Sparingly Soluble
0.1N HCl	Soluble	Soluble
0.1N NaoH	Soluble	Insoluble
Methanol	Freely Soluble	Soluble
Methanol (80%)	Soluble	Soluble
Acetonitrile	Soluble	Soluble
Acetate Buffer	Slightly soluble	Soluble

2.2.2 Selection of Mobile Phase

Initially to estimate Propranolol and Flunarizine in fix dosage form number of mobile phase in different ratio were tried. A result was shown in Table 6.2.

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 0.45□ filter paper to remove particulate matter and then degassed by sonication. Flow rate employed for analysis was 1.0 ml/min.[57]

Table 2.2: Mobile Phase Selection.

Mobile Phase	Ratio	Retention Time	
		PNL	**FNZ**
		Remark	
Water : Methanol	50 : 50 v/v	Poor Resolution	Not Found
		4.868	-
Water: Acetonitrile	50:50 v/v	Poor Resolution	Not Found
		5.555	-
ACN: Methanol	50 : 50 v/v	Most suitable	Most suitable
		3.256	5.658

2.2.3 Selection of Diluent

Diluent used for preparation of sample were compatible with mobile phase and no any significant affect retention and resolution of analyte. After various trials methanol was used as diluents.[58]

2.2.4 Selection of separation variable

Table 2.3: Separation Variable.

Variable	Condition
Column	-
Dimension.	250mm x 4.60mm
Particle Size	5□
Bonded Phase	Octadecylsilane (C_{18})
Mobile Phase	-
Methanol	50
Acetonitrile	50
Diluent	Methanol
Flow rate	1.0 ml/min
Temperature	Ambient
Sample Size	20 □l

Detection wavelength	250nm
Retention time	-
Propranolol	3.256 ± 0.2min.
Flunarizine	5.658± 0.4 min.

1. 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 1000 g/ml (Stock-A).

2. 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).

3. 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]

4. Linearity and Calibration Graph

To establish the linearity of analytical method, a series of dilution ranging from 10- 50 g/ml for PNL and 5-25 g/ml for FNZ were prepared. All the solution were filtered through 0.45 m 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]

Table 2.4: Linearity of PNL.

Standard Concentration g/ml	Area under Curve (AUC)						Mean
	Rep-1	Rep-2	Rep-3	Rep-4	Rep-5	Rep-6	
10	568.98	573.32	568.98	557.74	562.32	571.45	567.13
20	1070.5	1065.6	1055.4	1065.5	1074.6	1059.9	1065.3
30	1658.7	1650.2	1665.5	1650.2	1662.2	1655.7	1657.1
40	2174.6	2165.8	2169.9	2175.4	2160.5	2160.7	2167.8
50	2685.4	2670.6	2675.1	2165.8	2175.6	2160.3	2422.1
Correl Coeff							0.990
Slope (m)							50.01
Intercept (c)							62.92

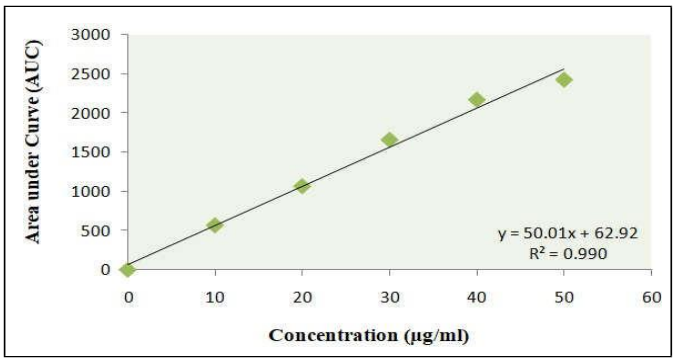

Figure 2.1: Calibration Curve of PNL.

Table 2.5: Linearity of FNZ.

Standard Conc. (g/ml)	Area under Curve (AUC)						Mean
	Rep-1	Rep-2	Rep-3	Rep-4	Rep-5	Rep-6	
5	255.658	259.856	250.654	263.325	249.985	260.325	256.634
10	512.226	518.856	517.456	520.145	519.987	510.224	516.482
15	765.589	756.651	760.887	769.985	759.965	753.328	761.068
20	1010.658	1015.658	1025.458	1005.698	1015.547	1015.658	1014.78
25	1258.854	1250.365	1247.789	1245.665	1240.698	1248.855	1248.7
Correl. Coeff (r^2)							0.999
Slope (m)							50.07
Intercept (c)							7.048

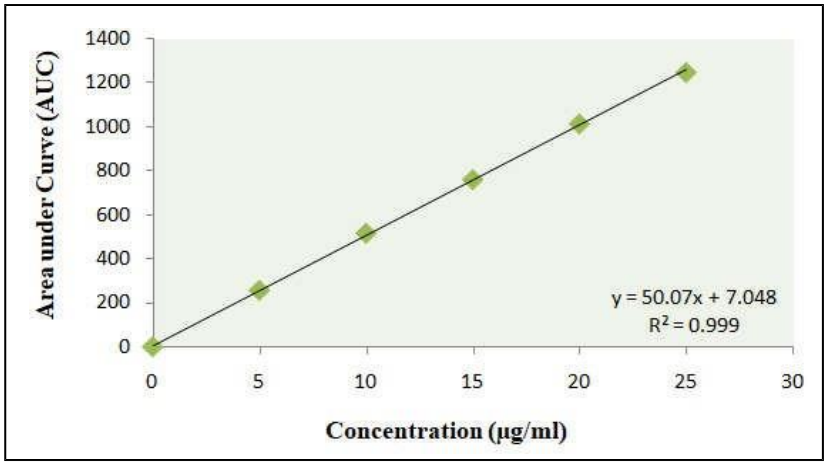

Figure 2.2: Calibration Curve of FNZ.

2.2.5 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 10□g/ml for PNL and 10□g/ml FNZ was injected separately. Peak report and column performance report were recorded for all chromatogram.

Table 2.6: System Suitability Parameters of PNL.

System suitability Parameter □	RT	AUC	No. of theoretical plates	Tailing factor
Rep-1	3.256	568.985	2565	1.15
Rep-2	3.258	573.325	2574	1.16
Rep-3	3.259	568.987	2565	1.17
Rep-4	3.257	557.745	2574	1.14
Rep-5	3.274	562.328	2574	1.16
Rep-6	3.278	571.458	2548	1.14
Mean	3.264	567.138	2566.667	1.153
S.D.	0.010	5.918	10.152	0.012

Table 2.7: System Suitability Parameters of FNZ.

System suitability Parameter □	RT	AUC	No. of theoretical plates	Tailing factor
Rep-1	3.256	568.985	2565	1.15
Rep-2	3.258	573.325	2574	1.16
Rep-3	3.259	568.987	2565	1.17
Rep-4	3.257	557.745	2574	1.14
Rep-5	3.274	562.328	2574	1.16
Rep-6	3.278	571.458	2548	1.14
Mean	3.264	567.138	2566.667	1.153
S.D.	0.010	5.918	10.152	0.012

2.2.6 Laboratory sample analysis

The commercial tablet formulation of PNL and FNZ is available in the strength of 10:75mg. Based on this different standard solutions were prepared for quantitative analysis, which gives satisfactory results. Stock solution was prepared in the same manner. Further dilutions were made to prepare the mixed standard of desired concentration.

Table 2.8: Laboratory Sample Analyses.

Standard Number	Concentration of PNL (□g/ml)	Concentration of FNZ (□g/ml)
1.	10	5
2.	20	10
3.	30	15
4.	40	20
5.	50	25

2.3 Validation of developed Method

The method was validated for the parameters reported below.[61-62]

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 regression equation and correlation coefficient of curve are given and the standard calibration curve of the drug is shown in figure 6.7 & 6.8. The response ratio (response factor) was found by dividing the AUC with respective concentration.

Table 2.9: Response Ratio Data for Linearity of PNL.

Concentration (□g/ml)	Mean AUC	Response Ratio
10	56.714	56.714
20	53.266	53.266
30	55.238	55.238
40	54.197	54.197
50	48.444	48.444
Mean		53.572
SD		2.808
%RSD		5.242

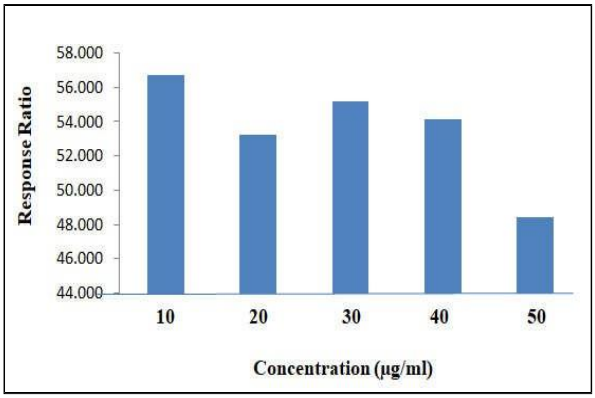

Figure 2.3: Response Ratio Curve of PNL.

Table 2.10: Response Ration Data for Linearity of FNZ.

Concentration (□g/ml)	Mean AUC	Response Ratio
5	256.634	51.327
10	516.482	51.648
15	761.068	50.738
20	1014.78	50.739
25	1248.7	49.948
Mean		50.880
SD		0.583
%RSD		1.146

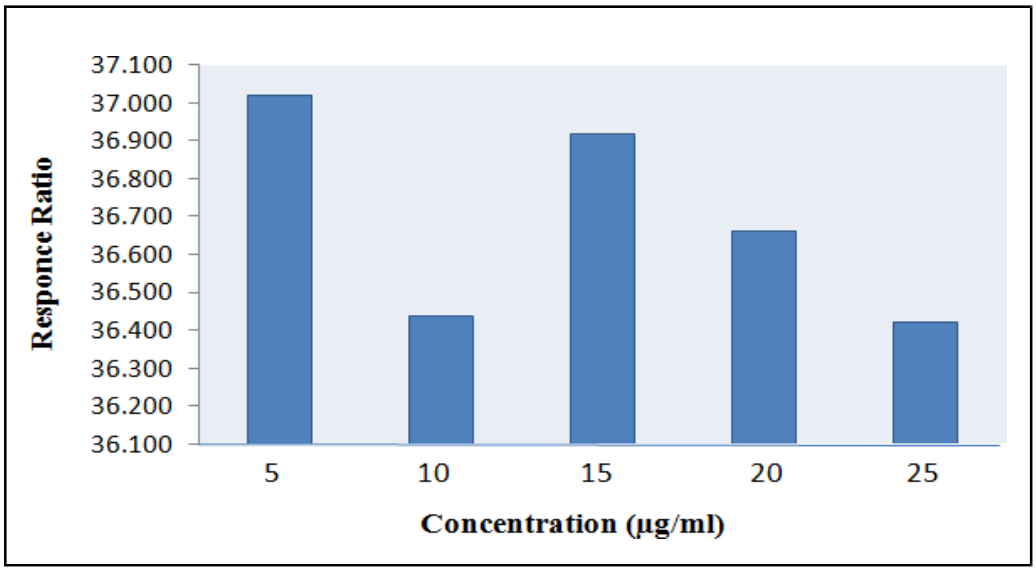

Figure 2.4: Response Ratio Curve of FNZ.

B. 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.

C. 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.

Table 2.13: Recovery study of PNL (120% Level).

Conc. of sample (□g/ml)	Amt. Added (□g/ml)	Conc. Found. (□g/ml)			% conc. Found			Mean % conc.
		Rep-1	Rep-2	Rep-3	Rep-1	Rep-2	Rep-3	
10	12	11.85	11.74	11.69	98.75	97.83	97.83	98.139
20	24	23.65	23.74	23.85	98.54	98.92	98.92	98.792
30	36	35.65	35.74	35.96	99.03	99.28	99.28	99.194
							MEAN	99.06
							SD	0.294
							% RSD	0.297

Table 2.16: Recovery Study of FNZ (120% Level).

Conc. of sample (□g/ml)	Amt. Added (□g/ml)	Conc. Found. (□g/ml)			% conc. Found			Mean % conc.
		Rep-1	Rep-2	Rep-3	Rep-1	Rep-2	Rep-3	
5	6	5.85	5.95	5.78	97.50	99.17	96.33	97.67
10	12	11.69	11.69	11.98	97.42	97.42	99.83	98.22
15	18	17.85	17.95	17.86	99.17	99.72	99.22	99.37
							MEAN	98.42
							SD	0.869
							% RSD	0.883

D. Precision

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

1. Repeatability

The repeatability was performed for five replicate at five concentrations in linearity range 1, 2, 3, 4 and 5□g/ml for PNL and 5, 10, 15, 20 and 25□g/ml for FNZ indicates the precision under the same operating condition over short interval time. Results of repeatability are reported in table respectively 6.17-6.18.

Table 2.17: Repeatability of PNL.

Conc.	Concentration Found (□g/ml)					
Rep.	10	20	30	40	50	
Replicate-1	9.95	19.95	29.65	39.85	49.85	
Replicate-2	9.98	19.85	29.85	39.78	49.78	
Replicate-3	9.85	19.65	29.78	39.65	49.95	
Replicate-4	9.69	19.97	29.65	39.98	49.78	
Replicate-5	9.98	19.87	29.78	39.74	49.96	
MEAN	9.89	19.858	29.742	39.8	49.864	
% MEAN	98.9	99.29	99.14	99.5	99.728	99.312
SD	0.124	0.127	0.089	0.124	0.088	0.110
% RSD	0.125	0.128	0.089	0.125	0.088	0.111

Table 2.18: Repeatability of FNZ.

Conc.	Concentration Found (□g/ml)					
Rep.	5	10	15	20	25	
Replicate-1	4.85	9.85	14.78	19.98	24.75	
Replicate-2	4.95	9.65	14.65	19.96	24.85	
Replicate-3	4.78	9.78	14.85	19.85	24.69	
Replicate-4	4.65	9.85	14.96	19.78	24.74	
Replicate-5	4.97	9.78	14.95	19.65	24.65	
MEAN	4.84	9.782	14.838	19.844	24.736	
% MEAN	96.8	97.82	98.92	99.22	98.944	98.341
SD	0.131	0.082	0.129	0.136	0.075	0.111
% RSD	0.135	0.083	0.130	0.137	0.076	0.112

Intermediate Precision

a) 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 reported in table 6.19-6.20 respectively.

Table 2.19: Day-to-Day variation of PNL.

Conc. Rep.	Concentration Found (□g/ml)					
	10	20	30	40	50	
Day 1	9.98	19.85	29.85	39.65	49.85	
Day 2	9.85	19.65	29.65	39.85	49.85	
Day 3	9.65	19.74	29.78	39.78	49.65	
MEAN	9.827	19.7467	29.76	39.76	49.7833	
% MEAN	98.267	98.7333	99.2	99.4	99.5667	99.033
SD	0.166	0.100	0.101	0.101	0.115	0.117
% RSD	0.169	0.101	0.102	0.102	0.116	0.118

Table 2.20: Day-to-day variation of FNZ.

Conc. Rep.	Concentration Found (□g/ml)					
	5	10	15	20	25	
Day 1	4.85	9.98	14.85	19.95	24.78	
Day 2	4.78	9.96	14.65	19.85	24.65	
Day 3	4.77	9.78	14.78	19.96	24.79	
MEAN	4.80	9.90	14.76	19.92	24.74	
% MEAN	96.00	99.06	98.40	99.6	98.96	98.405
SD	0.044	0.110	0.101	0.061	0.078	0.079
% RSD	0.045	0.111	0.103	0.061	0.079	0.080

E. Analyst to Analyst

Table 2.21: Analyst to analyst variation of PNL.

Conc. Rep.	Concentration Found (□g/ml)					
	10	20	30	40	50	
Analyst 1	9.95	19.78	29.85	39.78	49.85	
Analyst 2	9.85	19.69	29.69	39.65	49.96	
MEAN	9.900	19.735	29.77	39.715	49.905	
% MEAN	99.000	98.675	99.2333	99.2875	99.81	99.201
SD	0.071	0.064	0.113	0.092	0.078	0.083
% RSD	0.071	0.064	0.114	0.093	0.078	0.084

Table 2.22: Analyst to analyst of FNZ.

Conc. Rep.	Concentration Found (□g/ml)					
	5	10	15	20	25	
Analyst 1	4.98	9.95	14.85	19.98	24.95	
Analyst 2	4.85	9.99	14.78	20.01	24.89	
MEAN	4.943	9.980	14.877	19.997	24.947	
% MEAN	98.867	99.800	99.178	99.983	99.787	99.523
SD	0.092	0.028	0.049	0.021	0.042	0.047
% RSD	0.093	0.028	0.050	0.021	0.043	0.047

F. 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). Results of robustness are reported in table 6.23-6.24.

Table 2.23: Robustness of PNL.

Conc. Rep.	Concentration Found (□g/ml)					Mean
	10	20	30	40	50	
Replicate-1	9.95	19.85	29.85	39.98	49.85	
Replicate-2	9.85	19.65	29.96	39.78	49.96	
Replicate-3	9.68	19.74	29.85	39.65	49.85	
Replicate-4	9.78	19.78	29.74	39.78	49.78	
Replicate-5	9.85	19.68	29.78	39.74	49.63	
MEAN	9.822	19.74	29.836	39.786	49.814	
% MEAN	98.22	98.7	99.4533	99.465	99.628	99.093

SD	0.100	0.080	0.084	0.121	0.121	0.101
% RSD	0.102	0.081	0.084	0.121	0.122	0.102

Table 2.24: Robustness of FNZ.

Conc. Rep.	Concentration Found (□g/ml)					Mean
	5	10	15	20	25	
Replicate-1	4.98	9.98	14.95	19.98	24.85	
Replicate-2	4.99	4.99	14.85	19.95	24.78	
Replicate-3	4.85	5.01	14.95	14.96	24.96	
Replicate-4	4.95	4.99	14.96	14.85	24.65	
Replicate-5	4.96	4.95	14.85	14.98	24.96	
MEAN	4.955	6.653	14.927	17.453	24.867	
% MEAN	99.100	66.533	99.511	87.267	99.467	90.376
SD	0.056	2.234	0.057	2.758	0.131	1.047
% RSD	0.056	3.358	0.057	3.161	0.132	1.353

G. 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.

Table 2.25: LOD and LOQ of PNL and FNZ.

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

2.3.1 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 of tablet analysis are reported in table 6.26.

Table 2.26: 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

3. DISCUSSION

The developed methods were found to be linear (Table: 3.1). The values of mean percent recoveries were found to shown in Table: 3.2 and results of validation were shown in Table: 3.3. The mean percent label claims of tablets by the proposed methods were close to 100, indicating the accuracy of the proposed method and low values of standard deviation, percent coefficient of variation and standard error further validated the proposed method as shown in Table 3.1.

Table 3.1: 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

Linearity was established by least squares linear regression analysis of the calibration curve. The calibration curve was linear over the concentration range of 10-50µg/ml and 5- 25µg/ml, correlation coefficients were found to be 0.990 and 0.999 for Propranolol and Flunarizine respectively.

Table 3.2: 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

Recovery studies were carried out by applying the method to drug sample to which known amount of Propranolol and Flunarizine at three concentration levels of 80, 100 and 120 % were added. The results are given in Table 3.2.

Table 3.3: 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

The precision of the analytical method was studied by multiple sampling of the homogenous sample. The precision was done by measuring the absorbance for five times. The results are given in table 3.3.

Table 3.4: LOD and LOQ of PNL and FNZ.

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

Table 3.5: 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

Modern medicines for human use are required to comply with specific standards and regulation set forth by the concerned authorities. The efficacy and safety of medicinal products can only be assured by analytical monitoring of its quality. Pharmaceutical analysis is an art and science of determining the concentration of drug constituents present in marketed formulation. It is considered as an application of procedures necessary to determine and estimate the identity, strength, quality and purity of drug. Therefore, the quality control laboratory is considered as the backbone of the Pharma industries with ever-increasing need for the development of analytical techniques for drug formulation. In the present study, a successful attempt was made for the HPLC quantitative estimation of Propranolol and Flunarizine in bulk formulation. The method was developed by experimentation based on thorough literature survey and ascertained by statistical parameters of sampling. The entire work was performed on waters HPLC with U.V. Vis detector.

The result obtained shows the developed method to be precise, simple, rapid and accurate. Thus these can be used for routine analysis of Propranolol and Flunarizine in bulk drug and dosage form.

4. SUMMARY AND CONCLUSION

Modern medicines for human use are required to comply with specific standards and regulation set forth by the concerned authorities. The efficacy and safety of medicinal products can only be assured by analytical monitoring of its quality. Pharmaceutical analysis is an art and science of determining the concentration of drug constituents present in marketed formulation. It is considered as an application of procedures necessary to determine and estimate the identity, strength, quality and purity of drug. Therefore, the quality control laboratory is considered as the backbone of the pharma industries with ever- increasing need for the development of analytical techniques for drug formulation.

The developed methods were found simple, sensitive and economical for the simultaneous estimation of selected active pharmaceutical ingredients in their tablet formulation. Validation of developed methods was performed according to ICH guidelines. The standard deviation, % RSD for the methods are low, reflecting a high degree of precision of the methods. The results of the recovery studies performed show the high degree of accuracy of the proposed methods. The advantage of method was found being simple, economic, rapid and subsequently not required sophisticated technique, instrument and costly solvents. Thus, the proposed method can be successfully applied for determination and dissolution testing of selected drugs in commercial formulation.

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