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Research Article

METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF PROPRANOLOL HYDROCHLORIDE AND FLUNARIZINE DIHYDROCHLORIDE IN BULK AND PHARMACEUTICAL DOSAGE FORM BY RP-HPLC

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Abstract: A simple, efficient, reproducible RP-HPLC method for the simultaneous determination of Propranolol hydrochloride and Flunarizine dihydrochloride in bulk and pharmaceutical dosage form has been developed and validated**.** The seperation was carried out on Agilent xdb C18 (150mm×4.6mm I.D; particle size 5µm) column with a mobile phase consisting of methanol, acetonitrile and potassium dihydrogen phosphate (adjusted to pH 3.0 using orthophosphoric acid) in the ratio 20:20:60 respectively at a flow rate of 1.0 mL/min. Detection was carried out at 264nm. The retention times of Propranolol hydrochloride and Flunarizine dihydrochloride were found to be 2.058min and 6.708min respectively. The described method was linear over a concentration range of 10-60µg/mL and 5-30µg/mL. The proposed method was validated as per ICH guidelines. The developed method has adequate sensitivity, reproducibility and specificity for determination of Propranolol hydrochloride and Flunarizine dihydrochloride in bulk and its tablet dosage forms.

Key words: Flunarizine dihydrochloride , HPLC , Propranolol hydrochloride, Validation

INTRODUCTION

Propranolol hydrochloride (PRH): 1-[(1-methyl ethyl) amino] 3- (1-napthylenoylxy) 2 Propranolol hydrochloride (Fig-1**)** is a nonselective β blocker , that is, it blocks the action of epinephrine on both β1- and β2-adrenergic receptors. It is used for the treatment of angina pectoris, cardiac arrhythmia, hypertension, anxiety attacks, migraine prophylaxis and glaucoma.

Fig-1 : Chemical structure of Propranolol hydrochloride

Flunarizine dihydrochloride (**FNZ**): 1-[Bis (4-fluorophenyl) methyl]-4-[(2E)-3-phenylprop-2-enyl] piperazine dihydrochloride (Fig-2) is a calcium channel blocker which reduces arterial and arteriolar smooth muscle spasm by reducing intracellular Ca^{2+} overload due to brain hypoxia. It is used in migraine prophylaxis and also as antihistaminic and sedative.

Fig-2: Chemical structure of Flunarizine dihydrochloride

The combination of PRH and FNZ is used in the treatment of migraine prophylaxis[1]. Literature survey reveals a few spectrophotometric and bioanalytical methods for the estimation of both drugs as a single component and in combination with other drugs[2-9] HPTLC[10], spectrophotometric[11-12] methods have been already reported for simultaneous estimation of Propranolol hydrochloride and Flunarizine dihydrochloride in their combined dosage form. To our knowledge no method(s) have been reported for simultaneous estimation of both drugs in their combined dosage form by HPLC. Present study involves development and validation of HPLC method for the estimation of PRH and FNZ in combined dosage form.

EXPERIMENTAL METHOD

Instrumentation

Chromatographic separation was performed on JASCO 2080 model chromatograph equipped with an Agilent xdb-reverse phase C18 column (150 x 4.6 mm I.D; particle size 5 µm) was employed for the study. Sample injection was done with a Rheodyne 7725 injection valve via a 20 µL loop. Detection of the drug was done by using a UV-2075 detector (JASCO) and the output signal was monitored and integrated by JASCOBORWIN

software. UV spectra of PRH and FNZ combination was taken using a JASCO V-550 UV-Vis spectrophotometer in order to select the working wavelength for detection of the drugs. Solubility of the compound was enhanced by sonication on an ultrasonicator. Weighings was done on Digisum Electronic analytical balance (model DI 707).

Chemicals and reagents

Pharmaceutical grade Propranolol HCl and Flunarizine DiHCl working standards were obtained from Pellets pharma and Suraksha pharma Limited, Hyderabad, India. HPLC grade acetonitrile and methanol (Merck, Mumbai) solvents were used for preparing the mobile phase and the diluent. Sodium hydroxide and orthophosphoric acid are of analytical grade obtained from Sigma Aldrich. The commercially available tablets PROVANOL PLUS 10 containing a combination of these drugs (20 mg PRH and 10 mg FNZ) was purchased from local market.

Preparation of standard solution

Mixed standard solution was prepared by dissolving 50 mg of PRH and 25 mg of FNZ in 50 mL of methanol to get concentration of 1000µg/mL and 500µg/mL, respectively. Working standard solution was prepared by diluting 0.4mL of the above stock solution and transfers into a 10mL volumetric flask, and diluted up to the mark with diluents (methanol, acetonitrile and phosphate buffer in the ratio of 25:25:50). This will give the solution of Propranolol hydrochloride and Flunarizine dihydrochloride with concentration of 40 and 20µg/mL respectively. A 20µl injection of the above sample was performed and chromatographed.

Preparation of sample solution

Twenty tablets were weighed and average weight was determined and finely powdered. Tablet powder equivalent to 50 mg of propranolol hydrochloride and 25 mg of flunarizine dihydrchloride was accurately weighed and transferred to 50 ml volumetric flask. The contents were sonicated well and allowed to stand for 15 min with intermittent sonication to ensure complete solubility of drugs. The contents were made up to the mark with the methanol and filtered through a 0.45µ membrane filter. From the filtrate, dilution was made in a 10 mL volumetric flask with diluent to get 40µg/ml PRH and 20µg/ml FNZ respectively. Now the sample of 20µl was injected and chromatographed and the results from assay are summarized in Table-6.

Method development

A number of eluting systems containing water, methanol, aqueous buffer were used for separation of the drugs at initial development. Later, mixtures containing buffer, acetonitrile and methanol were used as eluting systems in different proportions like 35:20:45, 40:20:40 and 50:25:25 v/v. A mixture of methanol, acetonitrile and 20mM phosphate buffer[pH-3] in the ratio of 20:20:40 v/v provided an efficient separation of the drugs with good peak shapes and retention times. A flow rate of 1.0 mL/min was found to be optimum in the range of 0.5-1.0 mL/min which gave retention times of 2.058 min for propranolol hydrochloride and 6.708 min for flunarizine dihydrochloride with baseline stability.

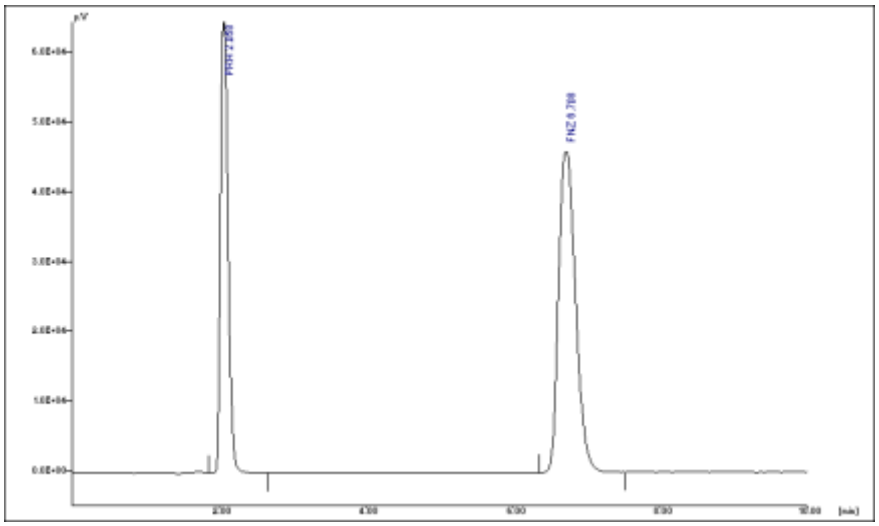

Fig 3: Chromatogram of standard solution of Propranolol HCl and Flunarizine DiHCl

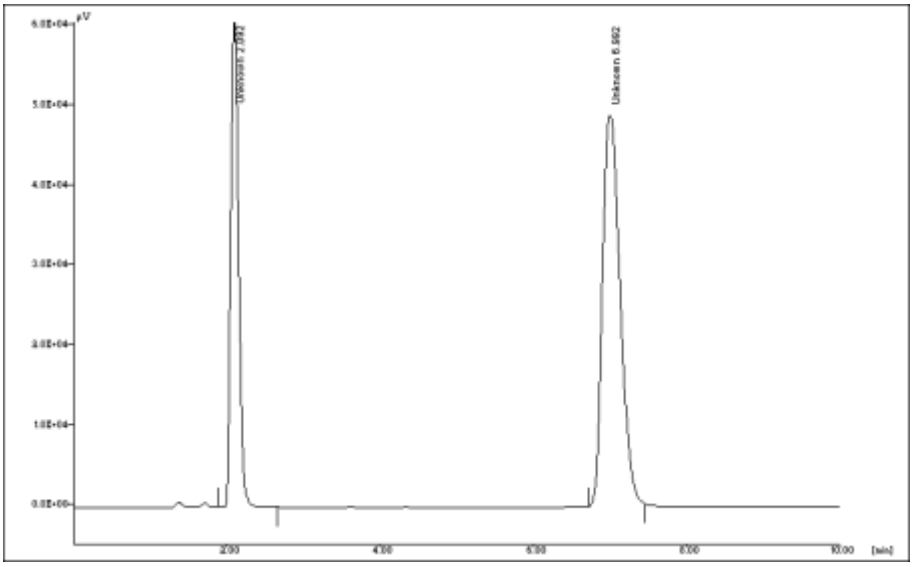

Fig 4: Chromatogram of PROVANOL PLUS 10[TABLET FORMULATION]

VALIDATION

The method was validated for accuracy, precision, linearity, limit of detection, limit of quantitation and robustness as per ICH[13] guidelines.

Specificity and selectivity

It is the extent to which the procedure applies to analyte of interest and is checked by examining the formulation samples for any interfering peaks. The specificity of the method was evaluated with regard to interference due to presence of excipients. The excipients used in formulation did not interfere with the drug peaks and thus the method is specific. The HPLC chromatograms recorded for the drug matrix (mixture of the drug and excipients) showed almost no interfering peaks within retention time ranges. Fig.3 and 4 show the representative chromatograms for standard and the formulation. The figures show that the selected drugs were clearly separated. Thus, the proposed HPLC method is selective.

Linearity

The linearity of the method was determined at six concentration levels ranging from 10 to 60 µg/ml of propranolol hydrochloride and 5 to 30 µg/ml of flunarizine dihydrochloride. The regression equation of calibration curves (Fig 5 & 6) were Y=10253x for PRH and Y=34732x for FNZ and are summarised in Table-1.

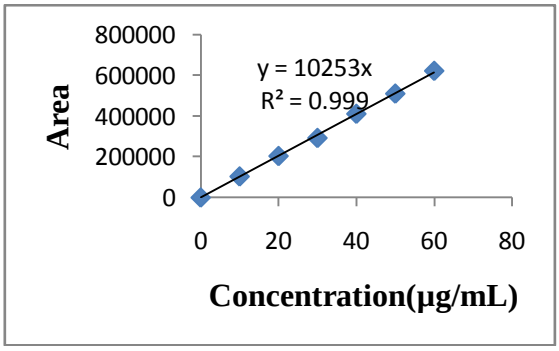

Fig-5: Calibration curve of Propranolol HCl

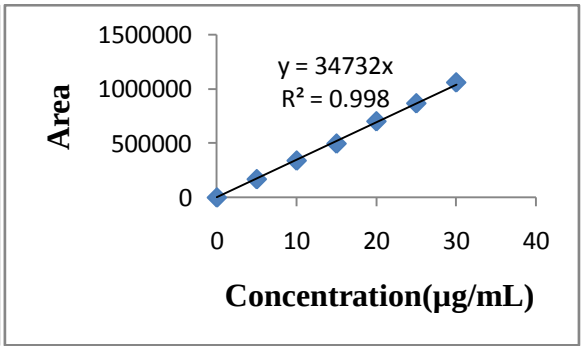

Fig-6: Calibration curve of Flunarizine DiHCl

Table 1: Linearity results of Propranolol HCl and Flunarizine DiHCl

Drug	Conc.(µg/ml)	Equation of regression line	R^2
PRH	10-60	Y=10253x	0.999
FNZ	5-30	Y=34732x	0.998

Table 2: Precision results of Propranolol HCl and Flunarizine DiHCl

Drug	%RSD(intraday)	%RSD(interday)
PRH	0.26	0.88
FNZ	0.23	0.92

Precision:

Precision of the method was studied as repeatability and intra-day and inter-day variations. The intra- and inter-day precision was determined by analyzing 20 µg/mL FNZ and 40 µg/ mL PRH, six times each on same day (intra-day study). This was repeated on the second day (inter-day study) and the results were shown in Table-2

Accuracy

The accuracy of the method was determined by recovery studies. The recovery studies were performed by standard addition method at 50%, 100%, 150% level for both the drugs i.e; three different levels corresponding to 20, 40 and 60 µg/mL for PRH and 10, 20 and 30 µg/mL for FNZ. Each level was repeated three times and the results are summarised in Table-3

Table 3: Accuracy results of Propranolol HCl and Flunarizine Di HCl

Analyte	Amount(%) of drug added to analyte	Theoretical conc.(ug/ml)	Measured conc.(ug/mi)	Recovery(%)	Rsd(%)
PRH	50	20	19.96	99.8	0.5
	100	40	40.1	100.2	0.05
	150	60	60.8	101.3	0.42
FNZ	50	10	9.96	99.6	1.1
	100	20	20.1	100.5	0.14
	150	30	30.6	102	0.97

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Limit of detection (LOD) and limit of quantitation (LOQ):

LOD is ability of analytical method to detect the lowest concentration of the analyte. LOQ is the lowest concentration of the analyte, which can be quantitatively analyzed with acceptable precision and accuracy. It can be calculated based on signal to noise ratio. Six replicates of the analyte were analyzed and quantified. The LOD of PRH and FNZ were 0.34µg/ml and 0.156µg/ml. The LOQ of PRH and FNZ were found to be 1.049 and 0.472µg/ml respectively.

Robustness:

Robustness of the method was determined by making slight changes change in flow rate, pH of buffer, and buffer concentration. It was observed that there were no marked changes in the retention time and area of the chromatograms which demonstrated that the RP HPLC method developed was robust and data are summarized in Table-4

Table 4: Robustness results of Propranolol HCl and Flunarizine Di HCl

	Propranolol hydrochloride			Flunarizine hydrochloride		
	RT*[a]	AF*[b]	TP*[c]	RT	AF	TP
Flow rate 0.9	2.3	1.26	63971	8.0	1.29	43505
Flow rate 1.1	1.9	1.28	55433	6.5	1.27	37861
pH 2.9	2.1	1.26	65171	6.9	1.26	45970
Ph 3.1	2.09	1.34	64958	7.0	1.26	44888
Buffer conc.15mM	2.05	1.29	65993	6.6	1.31	43778
Buffer conc.25mM	2.09	1.25	60343	6.8	1.36	37250

*a: retention time,*b: asymmetry factor, *c: theoretical plates

Table 5: System Suitability Parameters

Parameter	PRH	FNZ
Theoretical plates	413409.6	697452.4
HETP	64432	46111
Asymmetry	1.34	1.31
LOD (µg/ml)	0.34	0.156
LOQ (µg/ml)	1.049	0.472

Table 6 : Results of Assay from Tablet Dosage Form

Drug	Label claim (mg)	Amount found (mg)	%recovery
PRH	20	20.1	100.5
FNZ	10	9.9	99

System suitability

For system suitability, six replicates of the working standard sample were injected and the parameters like plate number (N), HETP and peak asymmetry of samples were calculated and the results are listed in Table-5

RESULTS AND DISCUSSION

The goal of this study was to develop a rapid HPLC method for analysis of propranolol hydrochloride and flunarizine dihydrochloride in its bulk and pharmaceutical formulations using a commonly used reverse phase C18 column. To develop an effective method for the analysis of the drugs, preliminary tests were performed in order to select adequate and optimum conditions. Parameters such as detection wavelength, ideal mobile phase and its combination, optimum pH and concentration of the standard solution were studied. To optimize the mobile phase, various proportions of buffer with acetonitrile and methanol were tested. Mobile phase containing a mixture of methanol, acetonitrile and buffer in the ratio of 20:20:60 v/v resulted in peaks with good shape and resolution. A flow rate of 1.0mL/min was found to be optimum in the 0.5-1.0 mL/min range resulting in the short retention time, baseline stability and minimum noise. By applying the proposed method, the retention times of PRH and FNZ were found to be 2.058 min and 6.708 min respectively. Quantitative linearity was obeyed in the concentration range of 10-60 and 5-30 µg/ml of PRH and FNZ respectively. The regression equations of concentration over their peak areas were found to be y=10253x (R^2=0.999), y=34732x (R^2=0.998) respectively where y is the peak area and x is concentration of PRH and FNZ (µg/ml). The number of theoretical plates obtained was 413409.6 and 697452.4 respectively which indicates the efficient performance of the column. The limit of detection and limit of quantitation were found to be 0.34 and 1.049 for Propranolol and 0.156 and 0.472 µg/ml for Flunarizine respectively, which indicates the sensitivity of the method. The high percentage recovery indicates that the proposed method is highly accurate. No interfering peaks were found in the chromatogram indicating that excipients used in tablet formulations didn't interfere with the estimation of the drug by the proposed HPLC method. The amount of PRH and FNZ present in the sample solutions were determined by fitting the responses into the regression equations of the calibration curve for PRH and FNZ respectively and the results obtained were comparable with the corresponding label claim (Table 6).

CONCLUSION

Proposed study describes a new RP-HPLC method for the estimation of propranolol hydrochloride and flunarizine dihydrochloride in combination using simple mobile phase. The method gives good resolution between the compounds with a short analysis time. The method was validated and found to be simple, sensitive, accurate, and precise.

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