IJPSR (2012), Vol. 3, Issue 06 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 20 February, 2012; received in revised form 12 March, 2012; accepted 25 May, 2012

DEVELOPMENT AND VALIDATION OF SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS DETERMINATION OF PROPRANOLOL HYDROCHLORIDE AND FLUNARIZINE DIHYDROCHLORIDE IN THEIR COMBINED DOSAGE FORMULATION

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Keywords:
Propranolol Hydrochloride,
Flunarizine Dihydrochloride,
Simultaneous Estimation,
UV Spectrophotometery

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ABSTRACT

A simple, accurate and precise spectrophotometric method has been developed for simultaneous estimation of Propranolol hydrochloride and Flunarizine dihydrochloride in combined dosage form. Simultaneous equation method is employed for simultaneous determination of Propranolol hydrochloride and Flunarizine dihydrochloride from combined dosage forms. In this method, the absorbance was measured at 289 nm for Propranolol hydrochloride and 253 nm for Flunarizine dihydrochloride. Linearity was observed in range of 24-64 µg/ml and 6-16 µg/ml for Propranolol hydrochloride and Flunarizine dihydrochloride respectively. Recovery studies confirmed the accuracy of proposed method and results were validated as per ICH guidelines. The method can be used for routine quality control of pharmaceutical formulation containing Propranolol hydrochloride and Flunarizine dihydrochloride.

INTRODUCTION: Propranolol Hydrochloride (PRP; 1-[(1-methyl ethyl) amino] 3- (1-napthylenoylxy) 2 Propranolol hydrochloride (**Figure 1**) is non-selective β-adrenergic antagonist and used in management of hypertension, angina pectoris, myocardial infarction and cardiac failure [1, 2].

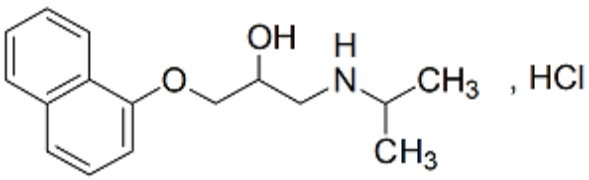

FIGURE 1: THE CHEMICAL STRUCTURE OF PROPRANOLOL HYDROCHLORIDE

Flunarizine Dihydrochloride (FLU; 1-[Bis (4-fluorophenyl) methyl]-4-[(2E)-3-phenylprop-2-enyl] piperazine dihydrochloride (**Figure 2**) is calcium channel blocker and used in migraine prophylaxis, epilepsy and vascular disease [3, 4].

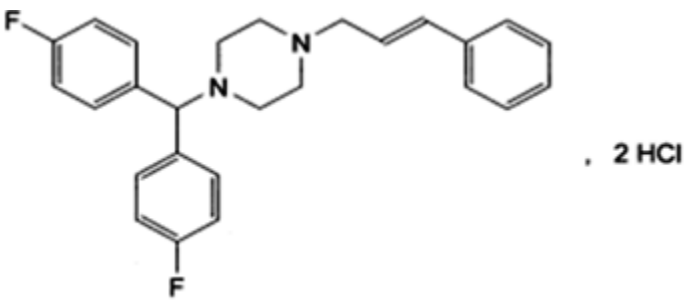

FIGURE 2: THE CHEMICAL STRUCTURE OF FLUNARIZINE DIHYDROCHLORIDE

The combination of these drugs (40 mg PRP and 10 mg FLU) has been recently approved for the treatment of migraine prophylaxis. The literature reveals that several titrimetric, spectrometric methods available for individual Propranolol Hydrochloride [5, 6, 7]. A number of

GC and HPLC method are reported for FLU determination in biological fluids [8, 9].

Only one Q-absorbance spectrometric method available for determination of Propranolol Hydrochloride and Flunarizine Dihydrochloride in Pharmaceutical Preparation [10], but no method has been reported for simultaneous estimation by U.V Spectrophotometric method in their combined tablet formulation.

MATERIALS AND METHODS:

Apparatus: A Shimadzu model 1700 (Japan) double beam UV/Visible spectrophotometer with spectral width of 2 nm, wavelength accuracy of 0.5 nm and a pair of 10 mm matched quartz cell was used to measure absorbance of all the solutions. Spectra were automatically obtained by UV-Probe system software (UV Probe version 2.31). An Electronic analytical balance (Acculab) and an ultrasonic bath were used in the study.

Reagents and Materials: Reference standard of PRP and FLU were obtained from Yarrow Chem. (Mumbai, India) and Esquire Pharmaceutics (Surendranagar, India) respectively as a gift sample, whereas their formulation obtained from local market. Analytical grade Methanol obtained from Finar Chemicals (Mumbai, India).

Preparation of Standard Stock Solution: An accurately weighed quantity of PRP (50 mg) and FLU (50 mg) were transferred to a separate 50 ml volumetric flask and dissolved and diluted to the mark with methanol. Take 10 ml of above solution into 100 ml volumetric flask and dilute the mark with distill water to obtain standard solution having concentration of PRP (100 µg/ml) and FLU (100 µg/ml). This solution was used as working standard solution.

Method: In simultaneous equation method, six working standard solutions having concentration 24, 32, 40, 48, 56, 64 µg/ml for PRP and 6, 8, 10, 12, 14, 16 µg/ml for FLU were prepared in distill water and measured the absorbance at 289 nm (λ_{max} of PRP) and 253 nm (λ_{max} of FLU), calculate absorptivity coefficients were calculated using calibration curve. The concentration of two drugs in the mixture can be calculated using following equations;

$$C_x = \frac{A_2 ay_1 - A_1 ay_2}{ax_2 ay_1 - ax_1 ay_2} \quad \text{..... (1)}$$

$$C_y = \frac{A_1 ax_2 - A_2 ax_1}{ax_2 ay_1 - ax_1 ay_2} \quad \text{..... (2)}$$

Where A_1, A_2 are absorbance of mixture at 289 nm (λ_1) and 253 nm (λ_2) respectively, ax_1 and ax_2 are absorptivities of PRP at λ_1 and λ_2 respectively ay_1 and ay_2 are absorptivities of FLU at λ_1 and λ_2 respectively, C_x and C_y are concentrations of PRP and FLU respectively.

METHOD VALIDATION [11]:

Linearity: The calibration curves were plotted over a concentration range of 24-64 µg/ml for PRP and 6-16 µg/ml FLU. Accurately measured standard stock solutions of each PRP (2.4, 3.2, 4.0, 4.8, 5.6 and 6.4 ml) and FLU (0.6, 0.8, 1.0, 1.2, 1.4 and 1.6 ml) were transferred to a series of 10 ml volumetric flask separately and diluted up to the mark with distill water. The absorbance of solution was then measured at 289 nm and 253 nm. The calibration curves were constructed by plotting absorbance versus concentration and the regression equations were calculated.

Precision:

- **Intraday Precision:** Mixed solutions containing 24-64 µg/ml PRP and 6-16 µg/ml FLU was analyzed 3 times on the same day and1 % RSD was calculated.
- **Interday Precision:** Mixed solutions containing 24-64 µg/ml PRP and 6-16 µg/ml FLUNA was analyzed on 3 different day and % RSD was calculated.

Accuracy: The accuracy of the method was determined by calculating recoveries of PRP and FLU in mixture by the standard addition method. Known amount of standard solutions of PRP (0, 16, 20 and 24 µg/mL) and FLU (0, 4, 5 and 6 µg/mL) were added to a pre-quantified sample solution of 20 µg/mL PRP + 5 µg/mL FLU mixture. The absorbance of PRP and FLU were recorded at λ_1 and λ_2. The percentage recovery was calculated by measuring the absorbance of both drug at their absorbance maxima and fitting these values into simultaneous equation. Each response was average of three determinations.

Limit of Detection and Limit of Quantitation: The limit of detection (LOD) and the limit of quantitation (LOQ) of the drug were derived by calculating the signal-to-noise ratio (S/N, i.e., 3.3 for LOD and 10 for LOQ) using the following equations designated by International Conference on Harmonization (ICH) guidelines.

$LOD = 3.3 \times \sigma/S$; $LOQ = 10 \times \sigma/S$

Where, σ = the standard deviation of the response and S = slope of the calibration curve.

Analysis of Tablet Dosage Form: Take 10 tablets and weighed. Find out average weight. Take tablet powder equivalent to 40 mg of PRP and 10 mg of FLU was transferred in 100 ml volumetric flask, dissolved and diluted up to mark with methanol. The solution was sonicated for 15minutes. Filter the solution through Whatman filter paper no. 42 and discard first few drops of filtrate. Pipette out 1ml of the above solution in 10ml volumetric flask and diluted to mark with simple distilled water. Absorbance of the resulting solution was measured at 289.0 nm and 253.0 nm against simple distilled water, relative concentration of two drugs in the sample was calculated using above equation (1) and (2).

TABLE 1: REGRESSION ANALYSIS DATA AND SUMMARY OF VALIDATION PARAMETERS FOR THE PROPOSED METHOD

Parameters	PRP	FLU
Wavelength range (nm)	289	253
Beer's law limit (µg/ml)	24-64	6-16
Regression equation (y = mx + c)	y = 0.019x + 0.108	y = 0.045x + 0.072
Slope	0.019	0.045
Intercept	0.108	0.072
Correlation Coefficient (r^2)	0.9992	0.9994
System Precision (%R.S.D)[a]		
1. Intraday Precision(n = 3)	0.53-0.89%	0.47-1.13%
2. Interday Precision(n = 3)	0.87-1.47%	1.29-1.79%
Accuracy (% recovery) (n = 3)	97.5-104.35%	96-102.7%
LOD[b] (µg/ml)	0.69	0.146
LOQ[c] (µg/ml)	2.1	0.444
Assay (±S.D.)[d] (n = 3)	94.06 ± 0.84	103.5 ± 1.47

[a]RSD = Relative standard deviation; [b]LOD = Limit of detection. [c]LOQ = Limit of quantitation; [d]SD is Standard deviation and n is number of replicates.

RESULT AND DISCUSSION: In simultaneous equation method, the primary requirement for developing a method for analysis is that the entire spectra should follow the Beer's law at all the wavelength, which was fulfilled in case of both these drugs. The two wavelengths were used for the analysis of the drugs were 289 nm (λ_{max} of PRP) and 253 nm (λ_{max} of FLU) at which the calibration curves were prepared for both the drugs. The overlain UV absorption spectra of PRP (289 nm) and FLU (253 nm) in distill water is shown in (**Figure 3**).

The validation parameters were studied at all the wavelengths for the proposed method. Accuracy was determined by calculating the recovery and the mean was determined (**Table 2**). The method was successfully used to determine the amounts of PRP and FLU present in the tablet dosage forms. The results obtained were in good agreement with the corresponding labeled amount (**Table 3**). Precision was calculated as repeatability and intra and inter day variations (% RSD) for both the drugs.

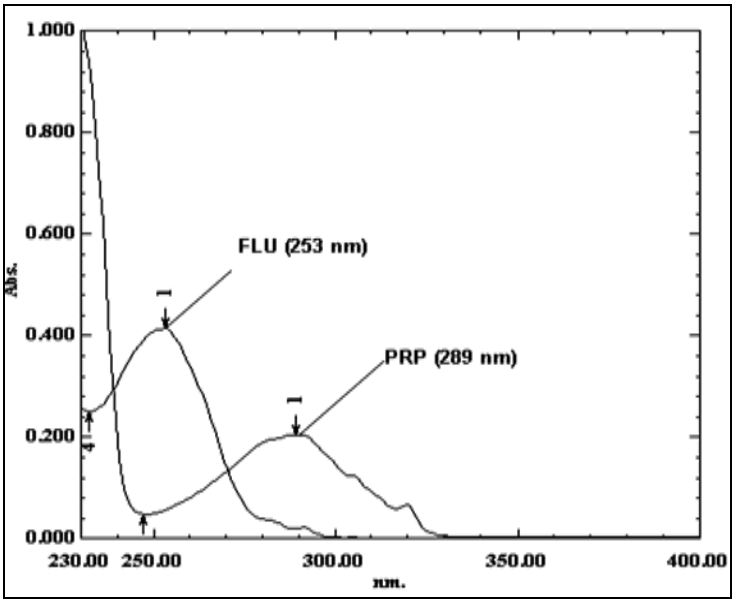

FIGURE 3: OVERLAIN ABSORPTION SPECTRA OF PRP (289 nm) AND FLU (253 nm) IN DISTILL WATER

TABLE 2: RECOVERY DATA OF PROPOSED METHOD

Drug	Level	Amount taken (µg/ml)	Amount added (µg/ml)	Amount added (%)	% Mean recovery (±S.D) (n = 3)
PRP	I	20	0	0	96 ± 1.7
	II	20	16	80	100.49 ± 1.74
	III	20	20	100	99.98 ± 1.18
	IV	20	24	120	101.35 ± 1.36
FLU	I	5	0	0	97.5 ± 1.56
	II	5	4	80	98.11 ± 0.43
	III	5	5	100	98.17 ± 0.86
	IV	5	6	120	101.44 ± 1.12

S.D is Standard deviation and n is number of replicates.

TABLE 3: ANALYSIS OF PRP AND FLU BY PROPOSED METHOD

Formulation	Labeled claim (mg)		Amount found (mg)		% Label claim (±S. D.) (n = 3)	
Provanol Plus-10 (Intas Product)	PRP	FLU	PRP	FLU	PRP	FLU
	40	10	38.6	10.35	94.06±0.84	103.53±1.47

S.D. is Standard deviation and n is number of replicates.

CONCLUSION: The developed simultaneous equation method is found to be simple, sensitive, accurate and precise and can be used for routine analysis of PRP and FLU. The developed method was validated as per ICH guidelines. Statistical analysis proved that the method is repeatable and selective for the analysis of PRP and FLU in their combined pharmaceutical formulations.

ACKNOWLEDGEMENT: The authors are thankful to Esquire Pharmaceutics, Surendranagar, Gujarat, India for providing gift sample of FLU for research. The authors are thankful to Shri Sarvajanik Pharmacy College, Mehsana, Gujarat, India for providing all the facilities to carry out the work.

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