Original Article

RP-HPLC method for simultaneous estimation of propranolol hydrochloride and flunarizine dihydrochloride in their combined dosage formulation

Abstract

Aim: A simple, precise and accurate RP-HPLC method with UV-Visible detector has been developed and subsequently validated for the simultaneous determination of propranolol hydrochloride (PRP) and flunarizine dihydrochloride (FLU) in their combined dosage formulation. **Materials and Methods:** The separation was based on the use of a Kromasil C8 analytical column (150 × 4.6 mm, i.d., 5 µm). The mobile phase consisted of a mixture of 70 volumes of methanol and 30 volumes of 10 mM phosphate buffer (pH 3.8). The separation was carried out at 40°C temperature with a flow rate of 0.8 ml/ min. **Result and Conclusion:** Quantitation was achieved with UV detection at 242 nm, with linear calibration curves at concentration ranges of 32–72 µg/ml for PRP and 8–18 µg/ml for FLU. The recoveries obtained were 98.97–101.10% and 98.86–102.27% for PRP and FLU, respectively. The method was validated according to the ICH guidelines in terms of linearity, accuracy, precision, specificity, robustness, limits of detection, limit of quantitation, and system suitability of analytical method validation.

Key words:
Flunarizine dihydrochloride, propranolol hydrochloride, RP-HPLC, validation

Introduction

Propranolol hydrochloride [PRP; 1-[(1-methyl ethyl) amino]-3-(1-napthylenoylxy)-2-propanol hydrochloride; Figure 1] is a non-selective beta adrenergic antagonist and used in the management of hypertension, angina pectoris, myocardial infarction, and cardiac failure.[1,2] Flunarizine dihydrochloride [FLU; 1-[Bis (4-fluorophenyl) methyl]-4-[(2E)-3-phenylprop-2-enyl] piperazine dihydrochloride; Figure 2] is a calcium channel blocker and used in migraine prophylaxis, epilepsy, and vascular disease.[3,4] The combination of these drugs (40 mg PRP and 10 mg FLU) has been recently approved for the treatment of migraine prophylaxis.[5] The literature reveals that several titrimetric, spectrometric methods are available for individual determination of PRP.[6-8] Gas chromatographic methods are reported for determination of FLU in biological fluids.[9] Only one Q-absorbance ratio method is reported for simultaneous determination of PRP and FLU in combined pharmaceutical dosage forms.[10] No single RP-HPLC method has been reported for simultaneous estimation of PRP and FLU their combined dosage formulation. In this work, an endeavor has been made to estimate both the drugs simultaneously by RP-HPLC method. Furthermore, method was validated as linearity, accuracy, precision, specificity, robustness, limits of detection, limit of quantitation, and system suitability of analytical method validation as per ICH guidelines.[11]

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Materials and Methods

Chemicals and reagents

Reference standards of PRP and FLU were kindly supplied by Yarrow Chem Ltd. (Mumbai, India) and Esquire Pharmaceutics (Surendranagar, India), respectively, as a gift sample. All reagents used were of HPLC grade, namely, methanol, water and ortho-phosphoric acid (80%) (Finar Chemicals Pvt. Ltd., Ahmedabad, India). Analytical grade

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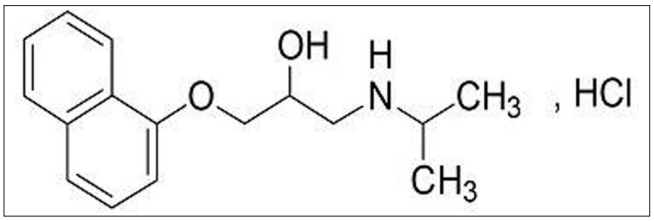

Figure 1: The chemical structure of propranolol hydrochloride

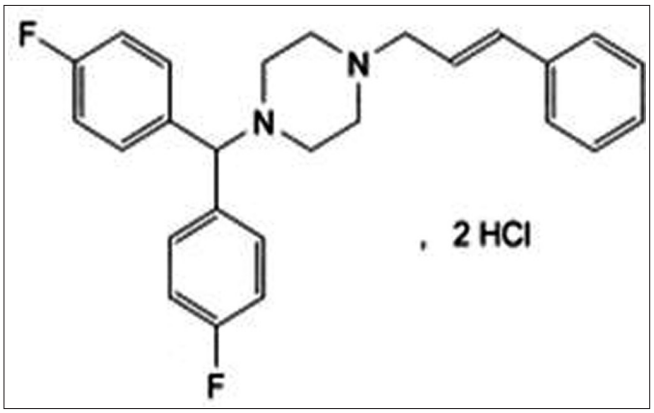

Figure 2: The chemical structure of flunarizine dihydrochloride

phosphate buffer was used to maintain the pH. Tablets of Provanol Plus 10 (Intas Pharmaceuticals Ltd., Ahmedabad, India) were procured from a local pharmacy.

Instrumentation

The chromatographic system, Shimadzu (model LC-2010$C_{H;}$ Shimadzu Corporation, Kyoto, Japan) equipped with auto-sampler, UV and Photodiode Array (PDA) detector, and Rheodyne injector with 20 µl loop volume. Weighing was done on a Digital Micro Balance an Acculab ALC 210.4 analytical balance and pH of buffer was maintained by pH analyser, Chemiline CL 180 µc based pH meter.

Chromatographic conditions

HPLC separations were performed on a Kromasil stainless steel C_8 analytical column (150 × 4.6 mm, i.d.) packed with 5 µm diameter particles. The mobile phase was a mixture of methanol and potassium dihydrogen phosphate buffer (pH 3.8) in a ratio of 70:30 (v/v). The pH of the buffer was adjusted to 3.8 with orthophosphoric acid solution (10%). The mobile phase was filtered through a Millipore membrane filter (0.45 µm) (Millipore, Milford, MA, USA) and was degassed before use. The flow rate was 0.8 ml/min. The detection was carried out at 242 nm for HPLC-UV detector.

Standard solutions

Stock solutions (1000 µg/ml) of PRP and FLU were prepared by dissolving separately 50 mg of each drug in 50 ml of diluent. These solutions were further diluted with the diluent to obtain working standard solutions of suitable concentrations (32, 40, 48, 56, 64, and 72 µg/ml for PRP and 8, 10, 12, 14, 16, and 18 µg/ml for FLU).

Sample preparation

Twenty tablets were weighed and powdered. An accurate weight of the powder equivalent to 20 mg of PRP and 5 mg of FLU was transferred into a 50-ml volumetric flask and extracted with 30 ml diluent in an ultrasonic bath for 30 min. The solutions thus prepared were diluted to volume and then filtered through a Whatman filter paper no-41. Suitable dilutions were made to prepare tablet solutions containing 40 µg/ml of PRP and 10 µg/ml of FLU. Solutions thus prepared were filtered using 0.45-mm filters (Millipore) then analyzed as mentioned under the construction of calibration graphs.

Results and Discussion

Optimization of chromatographic conditions

By the UV spectra of both drugs, wavelength may select 242 nm and show considerable absorbance for analysis. For separation of PRP and FLU with good resolution, various combinations of methanol, acetonitrile, and buffers were tried with different pH of buffer on C_{18} and C_8 column. Preliminary experimental trials indicate that use of different combinations of acetonitrile or methanol with buffer on C_{18} column was not able to separate the peaks of PRP and FLU. Finally, C_8 column was selected and phosphate buffer KH_2PO_4 was used for separation of PRP and FLU with suitable retention times and peak symmetry [Table 1]. Finally, a mobile phase consisting of methanol and phosphate buffer (KH_2PO_4) of pH 3.8 (adjusted with 10% solution of ortho-phosphoric acid) in a ratio of 70:30 v/v and a Kromasil C_8 column (150 mm × 4.6 mm i.d, 5 µm particle size) was selected to achieve good resolution and acceptable peak symmetry. Flow rates between 0.8 and 1.2 ml/min were tried. Flow rates 1 ml/min and 1.2 ml/min show PRP elute to early with retention time below 2 min and theoretical plate also below 2000, so 0.8 ml/min flow rate was selected to elute both the drugs within less than 10 min. The column temperature was set at 30°C which is the ambient room temperature.

Method validation

The developed method for simultaneous estimation of PRP and FLU has been validated in accordance with the ICH guidelines.[11]

Linearity

Linearity was checked by preparing standard solutions at six different concentration levels of 32, 40, 48, 56, 64, and 72 µg/ml for PRP and 8, 10, 12, 14, 16, and 18 µg/ml for FLU. Triplicate 20 µl injections were made for each concentration and were chromatographed under the chromatographic conditions mentioned above. Peak areas were plotted against the corresponding concentrations to obtain the calibration graph for each compound. The regression analysis data are given in [Table 2].

Table 1: Optimization of mobile phase

Stationary phase	Mobile phase	R_t		Comment
		PRP	FLU	
C18 (250 mm)	Methanol : 10 mM KH_2PO_4 (70 : 30)	1.90	2.40	Peak shape was not good and separation very poor
C18 (250 mm)	ACN : Methanol : 10 mM KH_2PO_4 (50 : 25 : 25)	6.92	12.66	Both peak obtained in 15 min but poor asymmetry
C8 (150 mm)	ACN : Methanol : 10 mM KH_2PO_4 (50 : 25 : 25)	5.46	9.82	In both peak splitting seen and also poor asymmetry
C8 (150 mm)	Methanol : 10 mM KH_2PO_4 [pH-7.0] (60 : 40)	25.4	15.50	Peak shape was not good and long run time
C8 (150 mm)	Methanol : 10 mM KH_2PO_4 [pH-4.0] (60 : 40)	2.90	6.89	Good resolution but slight broadening of PRP peak
C8 (150 mm)	Methanol : 10 mM KH_2PO_4 [pH-3.8] (70 : 30)	2.76	6.03	Good resolution, good peak shape of both peak, capacity factor and tailing factor acceptable

R_t retention time; PRP – Propranolol hydrochlorideand; FLU – Flunarizine dihydrochloride

Table 2: Linearity data of the developed method

Parameter	PRP	FLU
Linearity range (μg/ml)	32–72	8.0–18
Regression equation	$y = 6063x + 11142$	$y = 13456x - 3507$
Correlation coefficient (r^2)	0.999	0.999
Limit of detection (μg/ml)	2.44	0.43
Limit of quantification (μg/ml)	7.41	1.30

PRP – Propranolol hydrochlorideand; FLU – Flunarizine dihydrochloride

Sensitivity

The sensitivity of the analytical method was evaluated by determining the limits of detection (LOD) and quantitation (LOQ). The values of LOD and LOQ for PRP and FLU are given in [Table 2].

Accuracy

The accuracy of the method for assay determination was checked at three concentration levels of PRP and FLU: 80%, 100% and 120%. The percentage recoveries are tabulated in [Table 3]. The recovery was calculated from the slope and intercept of the calibration curve of each drug. As per ICH guideline, % recovery must between 98% and 102%.

Precision

Repeatability

System repeatability was determined by replicate applications and measurements of peak area for PRP and FLU. One dilution in six replicates was analyzed in the same day for repeatability, and results were found within acceptable limits (RSD <2) as shown in [Table 4].

Intermediate precision

Intermediate precision was assessed by the assay of sample sets on three different days (inter-day precision). Three dilutions in three replicates were analyzed and results were found within acceptable limits (RSD <2) as shown in [Table 4].

Robustness

As per ICH norms, small, but deliberate variations, by altering the pH or concentration of the mobile phase, were made to check the method capacity to remain unaffected. The developed mobile phase was methanol: phosphate buffer (pH 3.8) (70:30 v/v), so robustness of the method was done by changing in the pH of phosphate buffer 3.5 and 4.0. The change in the flow rate of mobile phase did not affect the peak area, and it may only change the retention time of peak of both the drugs. The results were found within acceptable limits (RSD<2) which are summarized in [Table 5].

Stability of sample solution

The sample solution stability was analyzed by injecting same solution at 0, 6, and 12 h. Identical change was not observed in the developed method. Also, the results were found within acceptable limits (RSD<2) which are summarized in [Table 6].

Specificity and selectivity

The specificity test of the proposed method was demonstrated that the excipients from sample do not interfere in the drug peak. It is shown in Figure 3 that excipients of tablet dosage form do not interfere with the analyte peak.

System suitability parameter

The retention times for PRP and FLU using optimum conditions were 2.74 and 6.74 min, respectively. For both compounds, the peak symmetries were <1.5 and the theoretical plates numbers were >2000. These values are within the acceptable range of USP definition and the chromatograms obtained under optimized chromatographic conditions. Figure 4 clearly shows the ability of the method to assess the analyte in the presence of matrix components. The results obtained are shown in Table 7.

Assay

The proposed method was also evaluated by the assay of PRP and FLU in their combined dosage formulation. The % assay was found to be 100.33% w/v for PRP and 102.98% w/v for FLU.

Conclusion

A simple precise, reliable, sensitive, and accurate RP-HPLC

Table 3: Result of recovery studies

Amt of sample (μg/ml)		Amt. of drug added (μg/ml)		Amt. found (μg/ml)		Amt. recovered (μg/ml)		% Recovery	
PRP	FLU	PRP	FLU	PRP	FLU	PRP	FLU	PRP	FLU
20	5.0	0.0	0.0	19.86	4.85	–	–	–	–
20	5.0	16	4.0	35.69	8.80	15.83	3.95	98.97	98.86
20	5.0	20	5.0	40.15	9.84	20.29	4.99	101.46	99.73
20	5.0	24	6.0	44.12	10.99	24.26	6.14	101.10	102.27

PRP – Propranolol hydrochlorideand; FLU – Flunarizine dihydrochloride

Table 4: Precision

Type of precision	% RSD	
	PRP	FLU
Repeatability	0.37	0.26
Intermediate precision	0.87	1.35

RSD – Relative standard deviation; PRP – Propranolol hydrochlorideand; FLU – Flunarizine dihydrochloride

Table 5: Robustness

Type of precision	% RSD	
	PRP	FLU
pH of mobile phase	0.19	0.15
Flow rate of mobile phase	0.88	0.65

RSD – Relative standard deviation; PRP – Propranolol hydrochlorideand; FLU – Flunarizine dihydrochloride

Table 6: Stability data of PRP and FLU

Hours	Peak area	
	PRP (40 μg/ml)	FLU (10 μg/ml)
0	254465	132551
6	256023	134439
12	256489	135285
Average	255659	134092
Std. deviation (SD)	1060	1400
%RSD	0.41	1.04

RSD – Relative standard deviation; PRP – Propranolol hydrochlorideand; FLU – Flunarizine dihydrochloride

Table 7: System suitability parameters

Parameter	PRP	FLU
Retention time (min)*	2.74±0.003	6.75±0.02
Number of theoretical plate*	2064±40.02	4313±20.23
Tailing factor*	1.44±0.05	1.3±0.044
HETP*	79.36±0.75	34.57±0.355

*Each value is the mean±SD of six determinations; PRP – Propranolol hydrochlorideand; FLU – Flunarizine dihydrochloride

method has been developed for the simultaneous determination of PRP and FLU. The developed method is suitable for the quantification of PRP and FLU in combined dosage form.

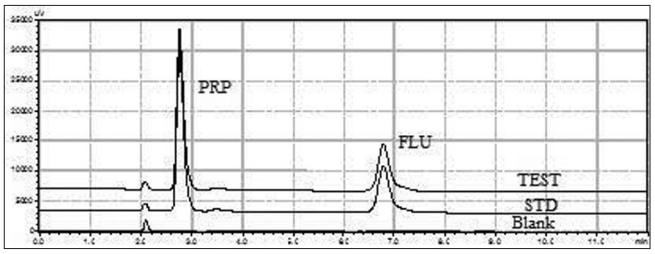

Figure 3: Specificity and selectivity chromatograms of PRP (40 μg/ml) and FLU (10 μg/ml)

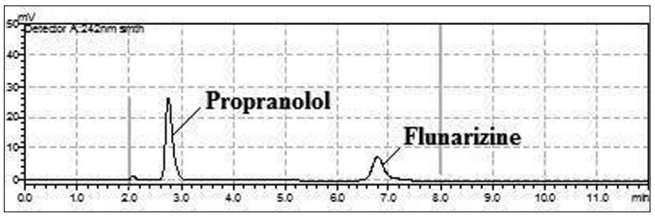

Figure 4: Chromatograms of PRP (40 μg/ml) and FLU (10 μg/ml) reference substances

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References

1. Moffat AC, Osselton MD, Widdop B, Galichet LY. Clarke's analysis of drugs and poisons. 3rd ed. London: Pharmaceutical Press; 2004. p. 1495-7.
2. In: Sweetman SC, editor. Martindale- The complete drug reference. 33rd ed. London: Pharmaceutical Press; 2002. p. 962-3.
3. Budavari S. The Merck Index – An Encyclopedia of Chemicals. Drugs and Biologicals, 12th ed. Merck & Co, Whitehouse Station, USA: 1996. p. 702.
4. Holmes B, Brogden RN, Heel RC, Speight TM, Avery GS. Flunarizine- A Review of its pharmacodynamic and pharmacokinetic properties and therapeutic use. Drugs 1984;27:6-24.
5. Cdsco.nic.in[Internet]. New Delhi:Central drug standard control organization; [updated 2010 March 16; cited 2012 April 06]. Available from: http://cdsco.nic.in/LIST%20OF%20APPROVED%20DRUG%20FROM%2001.htm
6. Basavaiah K, Chandrashekar U, Prameela HC, Nagegowda P. Titrimetric and spcetrometric determination of propranolol hydrochloride using chloramine-T. Indian Drugs 2004;41:303-5.
7. Basavaiah K, Chandrashekar U, Charan VS. Application of precipitation

and complexation reactions for the analysis of propranolol hydrochloride. Indian J Pharm Sci 2003;65:161-6.

8. Eman AA, Belal FF, Moustufa MA. Spectrophotometric determination of propranolol in formulation via oxidative coupling with 3-methylbenzothiazoline-2-one hydrazone. J Pharm Biomed Anal 2003;58:1179-86.
9. Kaptanovic IM, Torchin CD, Yonekawa WD, Kupfenberg HJ. Automated capillary gas chromatography determination of flunarizine. J. Chromatogr 1986;383:223-8.
10. Patil AS, Shirkhedkar AA, Surana SJ, Nawale PS. Q-Absorbance and multicomponent UV spectrophotometric method for simultaneous estimation of propranolol hydrochloride and Flunarizine Dihydrochloride in capsule. Der Pharma Chemica 2011;3:404-8.
11. The European Agency for the evaluation of medical products. ICH Topic Q2B, Note for guideline on validation of analytical procedure: Methodology, GPMP/ICH/254/1-18; 1996.

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