SIMULTANEOUS DETERMINATION OF PROPRANOLOL HYDROCHLORIDE AND FLUNARIZINE DIHYDROCHLORIDE IN BULK AND CAPSULE USING REVERSED – PHASE HIGH –PERFORMANCE THIN LAYER CHROMATOGRAPHY / DENSITOMETRY

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ABSTRACT

A simple, rapid and sensitive RP- HPTLC method has been established for the determination of Propranolol hydrochloride (PRH) and Flunarizine Dihydrochloride (FNZ) in bulk and capsule formulation. Separation of both these drugs were achieved on aluminum backed silica gel 60 RP-18 $F_{254}S$ HPTLC plates, prewashed with methanol using *methanol: toluene: ammonia (7:3:0.5 v/v)* as mobile phase. Densitometric scanning was performed at 267 nm. The R_f values for PRH and FNZ were found to be 0.63 and 0.48, respectively. The amount of PRH and FNZ estimated in capsule formulation were found to be 99.20 ± 1.04 and 98.89 ± 1.23, respectively. The accuracy of the method was found to be in the range of 99.30 – 100.41% for PRH and 99.56 -100.87 for FNZ. The method was validated as per ICH guidelines and can regularly used for analysis of PRH and FNZ in capsule dosage form.

Keywords: Propranolol hydrochloride, Flunarizine dihydrochloride, RP- HPTLC

INTRODUCTION

The combination of Propranolol hydrochloride(PRH) and Flunarizine Dihydrochloride(FNZ) is used in treatment of migraine[1].

PRH , 1-(isopropylamino)-3-(1-naphthyloxy)-2-propanol, is a non selective β blocker [2]. It is used in management of hypertension, angina pectoris, and cardiac arrhythmias [3].

Many analytical methods for determination of propranolol have been described such as HPLC [4], UV - spectrophotometric [5] and HPTLC [6] methods.

Flunarizine (FNZ), (*E*)-1-[Bis(4-fluorophenyl) methyl]-4-(3-phenyl-2-propenyl) piperazine[7], is difluorinated derivative of cinnarizine. It has antihistamine, sedative and calcium channel blocking activity [3]. Several analytical methods such as GC [8], HPLC [9], and UV - spectrophotometric [10] methods have been reported in bulk, pharmaceutical dosage form and in biological fluids for determination of FNZ.

For analysis of both these drugs in their capsule dosage form UV-spectrphotometric method has been reported [11].

To our notice, so far no HPTLC method has been reported for the simultaneous determination of PRH and FNZ in their combined dosage form.

Therefore, in the present communication an attempt has been made to develop a simple, rapid and precise RP - HPTLC method for simultaneous determination of PRH and FNZ in combined capsule dosage form and validation of developed RP-HPTLC method.

EXPERIMENTAL

Chemicals and Reagents

Pharmaceutical grade Propranolol Hydrochloride (purity, 99.80%) and Flunarizine Dihydrochloride (purity, 99.85%) working standards were obtained as generous gifts from Alkem Pharm., Mumbai, India. Methanol (A.R. Grade) was purchased from Merck Ltd., Worli, Mumbai, India. Capsule (BETACAP PLUS 10) was purchased from local market, containing PRH 40 mg and FNZ 10 mg per capsule.

Instrumentation

The plates were prewashed with methanol and activated at 110°C for 5 min, prior to chromatography. The linear ascending development was carried out in 20 × 10 cm twin trough glass chamber (Camag, Muttenz, Switzerland) using methanol: toluene: ammonia (7: 3 : 0.5*v/v*) as mobile phase, after saturation of the chamber with mobile phase vapor for 25 min. The development distance was 8 cm. After, chromatography plates were dried in a current of air with the help of air dryer. A Camag HPTLC system containing Camag Linomat 5 sample applicator, Hamilton syringe (100 µL), Camag TLC Scanner-3 with winCATS software version 1.3.0 and Camag twin- trough chamber (20 × 10 cm) were used for the present study. The source of radiation utilized was deuterium lamp emitting a continuous UV spectrum between 200 to 400 nm.

Preparation of standard solution

Mixed stock standard solution was prepared by dissolving 40 mg of PRH and 10 mg of FNZ in 50 mL of methanol to get concentration of 0.8 mg/mL and 0.2 mg/mL, respectively.

Selection of analytical wavelength

After chromatographic development bands were scanned over the range of 200 - 400 nm and the spectra were overlain; as good resolution and reproducible results were obtained when the both these drugs were estimated at 267 nm.

Preparation of calibration curves

From the mixed stock standard stock solution, 1- 6 mL was transferred into series of six volumetric flasks and volume was made up to the mark with methanol. From each volumetric flask a volume of 10 µL was applied on RP-HPTLC plate to obtain series of concentration 800 -4800 ng *per* band of PRH and 200 – 1200 ng *per* band of FNZ. The plates were developed and scanned as described under above established chromatographic conditions. Each standard in six replicates was analyzed and peak areas were recorded. Calibration curves of PRH and FNZ were plotted separately of peak area *vs.* respective concentration of PRH and FNZ**.**

Analysis of capsule formulation

The content of twenty capsules were accurately weighed and crushed into fine powder. A quantity of powder equivalent to 40 mg of PRH and 10 mg of FNZ was weighed and transferred to a 50 mL volumetric flask containing approximately 25 mL of methanol, ultrasonicated for 5 min, and volume was made up to the mark with the methanol. The solution was filtered through Whatman 41 filter paper, and 3 mL of filtrate was further diluted to 10 mL with methanol. Then, volume of 10 µL was applied to a TLC plate. After chromatographic development the peak areas of the bands were measured at 267 nm and the amount of each drug present in sample was estimated from the respective calibration curves. Procedure was repeated six times for the analysis of homogenous sample.

RESULTS AND DISCUSSION

Mobile phase optimization

Mobile phases containing various ratios of methanol, toluene i.e. 8:2 *v/v* and 7:3 *v/v* were tried to give compact spot for FNZ and for PRH tailing of spot was observed. To rule out the problem, small proportion of ammonia was added. Finally, the mobile phase containing methanol: toluene: ammonia (7:3:0.5, *v/v*) was selected as optimal for obtaining well defined and resolved peaks. The optimum wavelength for detection and quantitation used was 267 nm **(figure 1).** The retention factors for PRH and FNZ were found to be 0.63 ± 0.03 and 0.48 ± 0.03, respectively. Representative densitogram obtained from a mixed standard solution of PRH and FNZ is shown in (**Figure 2)**.

Validation

The method was validated by establishing linearity, robustness, ruggedness, specificity, accuracy, inter day and intra- day precision of measurement of sample application. The limit of detection and limit of quantification were also determined.

Calibration curve

Linearity was studied in the concentration range from 800 to 4800 ng *per* band for PRH and 200 to 1200 ng *per* band for FNZ. Both the drugs showed good linearity in the tested range. The regression equations for PRH and FNZ were found to be y = 2.052x + 1397 and y = 5.253x + 868.4. The regression

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co-efficient (r^2) value for PRH and FNZ was found to be > 0.997

Robustness and Ruggedness

Robustness of the method was determined by small considerable changes in mobile phase volume, mobile phase composition, development distance and chamber saturation period. Robustness of the method was performed at a concentration level of 2400 and 600 ng *per* band for PRH and FNZ. When very small changes were made to the method conditions there were no marked changes in chromatographic behavior and content of the drug, as evident from the low value of percentage RSD indicating the method is robust. **(Table 1)**

Ruggedness test was determined between two different analysts. The value of percentage RSD was below 2.0 %, showed ruggedness of developed analytical method.

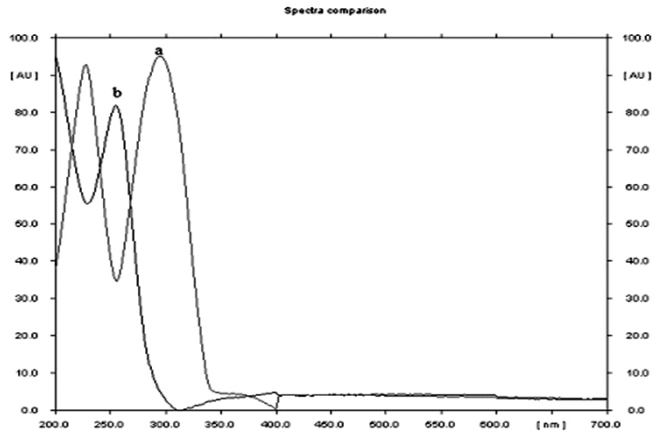

Figure 1: Typical overlain spectra of PRH (a) and FNZ (b) standard solutions.

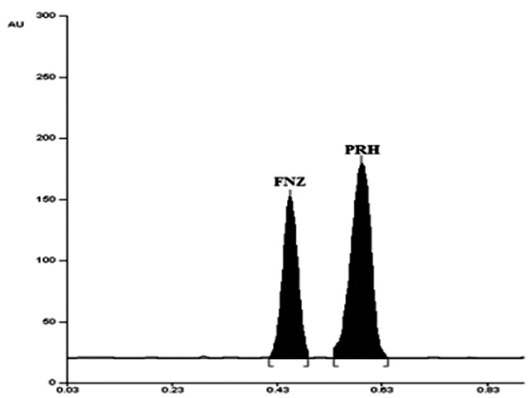

Figure 2. Densitogram of FNZ (400 ng *per* band, Rf 0.48 ± 0.03) and PRH (1600ng *per* band, Rf 0.63 ± 0.03) in methanol: toluene: ammonia (7: 3: 0.5 *v/v*)

Table 1: Robustness studies.

Parameters	PRH	FNZ
	% RSD (n = 6)	%RSD
Mobile phase volume	1.20	1.35
Mobile phase composition	0.77	1.49
Development distance	0.84	1.42
Duration of saturation	0.91	1.34

Specificity

There was no interference from sample placebo in peak purity of PRH and FNZ. It showed that developed method was specific for the analysis of PRH and FNZ in capsule dosage form.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD and LOQ were calculated using equations; LOD = 3.3 x N/B and LOQ = 10 x N/B, where, N is standard deviation of the peak areas of the drugs (n=3), taken as a measure of noise, and 'B' is the slope of the corresponding calibration curve. The LOD and LOQ for PRH were found to be 24.84 ng and 75.30 ng, respectively. For FNZ, LOD and LOQ were found to be 15.91 ng and 48.23 ng , respectively

Precision and Accuracy

Precision of the method was studied as repeatability and intra-day and inter-day variations. The repeatability of sample application and measurement of peak area was determined by performing six replicate measurements of 2400 ng/band for PRH and 600 ng/band for FNZ; the effects on the results were studied in terms of %RSD and found to be less than 2.

Intra-day variation was determined by analyzing three different concentrations for three times within a day and Inter-day precision was assessed by three different concentrations for three different days, over a period of week.

The intra-day and inter-day variation were measured at three different concentrations 1600, 2400, 3200 ng/band (PRH) and 400, 600, 800 ng/band (FNZ). The effects on results of intra-day and inter-day variations were assessed in terms of %RSD; found to be less than 2.

The accuracy of the experiment was established by spiking pre-analyzed sample with known

amounts of the corresponding drugs at three different concentration levels i.e. 80, 100 and 120 %

of the drug in the capsule. The spiked samples were then analyzed for six times. The mean recovery is within acceptable limits, indicating the method is accurate. (**Table 3).**

The low values of %RSD indicative of the method is precise and accurate.

Table 3: Recovery Studies.

Compo-nents	Initial Amount (*ng per* band)	Sample level Added (%)	Amount recovered ± S.D.[*ng per* band] n=3	% Recov-ered	% RSD
PRH	1600	80	1273±13.49	99.52	1.05
	1600	100	1606±20.61	100.41	1.28
	1600	120	1906±14.45	99.30	0.75
FNZ	400	80	322.81±4.48	100.87	1.38
	400	100	399.34±5.80	99.83	1.45
	400	120	477.89±3.06	99.56	0.64

n- number of determinations

Different validation parameters for RP- HPTLC method are summarized in (**Table 4).**

Table 4: Summary of validation parameters.

Parameter		
	PRH	**FNZ**
Linearity Range [ng *per* band]	800 - 4800 ng	200 - 1200 ng
Correlation coefficient	0.997	0.997
Limit of detection [ng]	24.84	15.91
Limit of quantification [ng]	75.30	48.23
Precision (%RSD)		
Intra-day[n = 6]	0.37-1.11	0.65 -1.48
Inter-day[n = 6]	0.57 - 1.31	0.79 - 1.95
Repeatability [n = 6]	1.68	1.88
Ruggedness [%RSD]		
Analyst 1 [n = 6]	1.50	1.73
Analyst 2 [n = 6]	0.90	1.40

Analysis of capsule formulation:

Using the proposed chromatographic method, assay of PRH and FNZ in their capsules (BETACAP PLUS 10, label claim: 40 mg PRH and 10 mg FNZ per capsule) was carried out. The peaks at R_f 0.63 for PRH and *Rf* 0.48 for FNZ were observed in the densitogram of the drug samples extracted from capsules. There was no interference from the excipients commonly present in the capsules. The drug content was found to be 99.20% and 98.89% for PRH and FNZ, respectively.

CONCLUSION

The proposed method is simple, accurate, cost effective, less time consuming. Statistical analysis proved that the method is reproducible and efficient for the simultaneous estimation of PRH and FNZ as bulk drugs and in combined pharmaceutical dosage forms without any interference from the excipients.

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