Development and Validation of a HPTLC Method for Simultaneous Quantitation of Flunarizine Dihydrochloride and Propranolol Hydrochloride in Capsule Dosage Form

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A simple, precise, accurate, and rapid high-performance thin layer chromatographic method has been developed and validated for the simultaneous quantitation of flunarizine dihydrochloride and propranolol hydrochloride in a combined capsule dosage form. The method was carried out on precoated silica gel 60 F_{254} TLC aluminum plate, (20×10 cm^2). The solvent system was ethyl acetate:methanol:glacial acetic acid in the proportion of 8:1:1, (v/v/v). R_f value for flunarizine dihydrochloride and propranolol hydrochloride was found to be 0.62±0.02 and 0.18±0.02, respectively. The linearity regression analysis for calibration showed 0.999 and 0.999 for flunarizine dihydrochloride and propranolol hydrochloride with respect to peak area and height in the concentration range of 50-350 ng/spot and 500-3500 ng/spot, respectively. Accuracy of recovery studies was found to be 98-100.28 and 99.11-99.45% for flunarizine dihydrochloride and propranolol hydrochloride, respectively. The amounts of drug in marketed formulation were 100.5 and 101.25% of flunarizine dihydrochloride and propranolol hydrochloride, respectively. The method developed can be used for routine analysis in bulk drug and capsule dosage form.

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Flunarizine dihydrochloride (FLU) a calcium channel blocker chemically is (E)-1-[Bis (4-fluorophenyl) methyl]-4-(3-phenyl-2-propenyl) piperazine dihydrochloride (fig. 1). It 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. Flunarizine dihydrochloride is official in British Pharmacopoeia (BP)[1]. Propranolol hydrochloride (PRO) a non-selective beta blocker, chemically is (2RS)-1-[(1-methylethyl) amino]-3-(1-naphthalenyloxy)-2-propanolhydrochloride (fig. 1). PRO 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. PRO is official in Indian Pharmacopoeia (IP)[2]. FLU and PRO are used in combination for migraine prophylaxis[3,4]. Literature survey reveals that various analytical methods like UV[5,6], HPLC[7-12], HPTLC[13], and GC[14] are reported for the individual drugs and in combination with others and one paper on UV using methanol as solvent for simultaneous estimation of FLU and PRO[15]. However, no method is reported for simultaneous estimation of these two drugs by HPTLC. The aim of the present investigation was to develop a simple, precise, and accurate HPTLC method for determination of FLU and PRO in bulk drug and sustained release capsule dosage form. The method was validated in compliance with ICH guidelines[16].

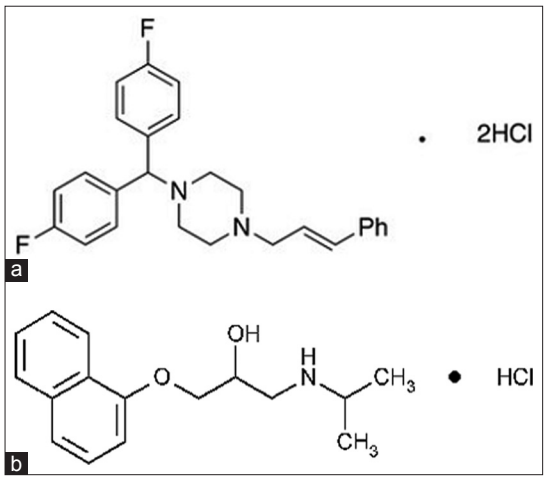

Fig. 1: Structure of anlytes.
Structures of (a) flunarizine dihydrochloride (FLU) and (b) propranolol hydrochloride (PRO).

FLU was gifted by FDC India Ltd. Jogeshwari, Mumbai, India and PRO was procured from Shreepati Pharmaceuticals Pvt. Ltd. Indore, India and used without any further purification. Ethyl acetate, methanol, and glacial acetic acid (A. R. Grade) were purchased from Merck Specialities Pvt. Ltd., Mumbai, India. Marketed formulation (Betacap Plus 10 capsules) was purchased from local market, containing FLU 10 mg and PRO 40 mg per capsule.

A Camag TLC system (Muttens, Switzerland) comprising of Camang automatic TLC sampler 4 (ATS4) applicator, syringe capacity 25 µl, Camang TLC scanner 4, Camag WinCATS software version 1.4.6.2002, Camag twin trough chambers (10×10 cm^2 and 20×10 cm^2), Camag TLC visualizer, ultrasonicator and centrifuge was used during the study. TLC plates used were precoated silica gel aluminium plate 60 F_{254}, 20×10 cm^2 with 0.2 mm thickness (E. Merck, Mumbai, India).

The chromatographic conditions maintained were precoated silica gel on aluminum plate 60 F_{254}, (20×10 cm^2 and 10×10 cm^2, prewashed by methanol and activated at 60° for 5 min prior to chromatography) as stationary phase, ethyl acetate:methanol:glacial acetic acid in the proportion of 8:1:1, (v/v/v) as mobile phase for the FLU and PRO. Ten millilitre volume of mobile phase in twin trough chamber with 10 min as chamber saturation time at room temperature (30±1°) and RH (60±5%). Application rate of 0.1 µl/s, scanner band width 8 mm, and distance between two bands is 10 mm.

Detection was done densitometrically in the reflectance-absorption mode, keeping slit dimension at 6×0.45 mm and scanning speed of 20 mm/s using a UV detector at 262 nm for both APIs. Standard stock solutions of FLU and PRO were prepared by accurate weighing of 10 mg for both the drugs in the separate 10 ml volumetric flask and dissolving in a methanol and then made up to mark with methanol. For simultaneous quantitative studies of both drugs, working standard solution of both the drugs was prepared by appropriate dilution of standard stock solutions to get 0.025 µg/µl of FLU and 0.25 µg/µl of PRO.

Twenty capsules were weighed accurately and ground to fine powder. Weights equivalent to 10 mg of FLU and 40 mg of PRO were transferred to volumetric flasks and mixed with 5 ml methanol. The solution was sonicated for 20 min. The solution was centrifuged for 25 min and supernatant collected. The concentrations obtained were 2 µg/µl of FLU and 8 µg/µl PRO, respectively.

For HPTLC analysis, initially various mobile phases were tried in attempts to obtain the best separation and resolution between FLU and PRO. The TLC plates were prewashed with methanol. Activation of plates was done in an oven at 60° for 5 min. The chromatographic conditions maintained were precoated silica gel $60F_{254}$ aluminum sheets (20×10 cm^2) as stationary phase, migration distance allowed was 8 cm. The mobile phase consisting ethyl acetate:methanol:glacial acetic acid in the proportion of (8:1:1, v/v/v) was selected that gave satisfactory separation and two well resolved peaks for FLU and PRO at 262 nm (fig. 2). As FLU and PRO exhibit significant absorbance at wavelength 262 nm which was selected by scanning standard solutions of both drugs over 200 nm to 400 nm wavelength. The R_f value for FLU and PRO was 0.18±0.02 and 0.62±0.02, respectively.

The developed method for FLU and PRO was validated using the following parameters. The linearity was determined for both drugs FLU and PRO separately by plotting a calibration graph of peak area against their respective concentration with the help of winCATS software. Each reading was the average of three determinations. From the calibration curve, it was found that FLU shows linearity in the range of 50 to 350 ng/spot, whereas PRO shows in the range of 500 to 3500 ng/spot. The regression coefficient for both the APIs are shown in Table 1.

Accuracy of the developed method was confirmed by doing a recovery study as per ICH guidelines at three different concentration levels (80, 100, and 120%) by replicate analysis (*n*=3). Recovery studies of FLU and PRO were performed by standard addition method. For that known amounts of standard solutions of FLU and PRO (4.6, 6, and 7.2 µl), were added to pre quantified sample solutions of capsule dosage forms and then percentage of drug content was calculated. The results of the recovery studies are reported in Table 2.

TABLE 1: LINEAR REGRESSION DATA FOR CALIBRATION CURVE

Parameters (units)	FLU	PRO
Linear range (ng/spot)	50-350	500-3500
Slope	6.561	1.091
Intercept	155.9	147.6
Regression coefficient	0.9995	0.9999

FLU=Flunarizine dihydrochloride, PRO=Propranolol hydrochloride

TABLE 2: SUMMARY OF VALIDATION PARAMETERS OF HPTLC

Parameters (units)	FLU	PRO
Recovery (%)	98-100.28	99.11-99.45
Precision (%RSD)		
Repeatability (*n*=9)	0.70	1.12
Intermediate precision		
Intra-day (*n*=3)	0.69-1.02	0.09-1.59
Inter-day (*n*=3)	0.07-1.06	0.09-0.76
LOD (ng/spot)	3.1	12.05
LOQ (ng/spot)	9.4	57.96

FLU=Flunarizine dihydrochloride, PRO=Propranolol hydrochloride, LOD=Limit of detection, LOQ=Limit of quantitation, RSD=Relative standard deviation.

Precision of the method was verified by repeatability and intermediate precision studies. The standard deviation and relative standard deviation were calculated for two drugs for repeatability (*n*=9) at concentration 300 ng and 3000 ng for FLU and PRO, respectively. Intermediate precision was carried out by intra- and inter day precision studies. Intra-day precision of the method were evaluated for mixtures of FLU and PRO by repeatedly injecting (*n*=3) at three different independent concentrations, i.e., 100, 150, and 200 ng/spot for FLU and 1000, 1500, and 2000 ng/spot for PRO without changing the position of the plate. Inter-day precisions of the proposed method were determined by estimating the corresponding responses three times on the three different days for three different concentrations, i.e., 100, 150, and 200 ng/spot for FLU and 1000, 1500, and 2000 ng/spot for PRO. The % RSD values are shown in Table 2.

The proposed validated method was successfully applied to determine FLU and PRO in their combined sustained release capsule dosage form. From the above sample stock solution 0.1 and 0.2 µl were applied on pre-washed TLC plate, developed in the above mobile phase, dried in air and densitometrically analyzed as described above. From the peak area obtained in the chromatogram, the amounts of all the drugs were calculated and results of assay are shown in Table 3.

TABLE 3: ASSAY RESULTS FOR THE COMBINED DOSAGE FORM

Drug	Amount (mg)	Amount found (mg)	Label claim (%)±SD
FLU	10	10.03	100.33±0.28
PRO	40	40.24	100.62±0.62

FLU=Flunarizine dihydrochloride, PRO=Propranolol hydrochloride

The proposed HPTLC method was optimized with several solvent systems. The mobile phase ethyl acetate:methanol:glacial acetic acid (8:1:1, v/v/v) gave good resolution with R_f values of 0.18±0.02 and 0.62±0.02 for FLU and PRO, respectively. Peak resolution for mixture of standard APIs was obtained with clear baseline separation (fig. 2) and for marketed formulation (fig. 3). The calibration curves for FLU and PRO were constructed by plotting area *vs*. concentration. The validation parameters were studied for proposed method. The method was found to be accurate with percent recovery of 98-100.28 and 99.11-99.45% for FLU and PRO, respectively. The method was found to be precise with RSD of 0.69-1.02 for intraday (n=3) and 0.07-1.06 for interday (n=3) for FLU and 0.09-1.59 for intra-day (n=3) and 0.09-0.76 for inter-day (n=3) for PRO. The LOD for FLU and PRO were found to be 3.1 ng/sopt and 9.6 ng/spot, respectively, while LOQ were 12.05 and 57.96 ng/spot, respectively. Summary of validation parameters is shown in Table 3. The assay results for marketed formulation were 100.5 and 101.25% of FLU and PRO, respectively. The results of analysis of pharmaceutical dosage forms by the proposed methods are highly reproducible and reliable and are in good agreement with the label claim of the drug. The additives usually present in the pharmaceutical formulations of the assayed samples did not interfere with the determination of FLU and PRO. The method is simple, precise, specific, and accurate and can be used for the routine simultaneous analysis of the FLU and PRO in pharmaceutical preparations.

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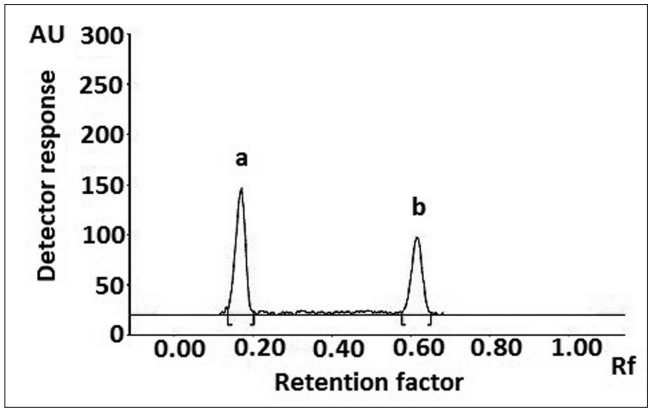

Fig. 2: HPTLC chromatogram of standard mixture.
HPTLC chromatogram of (a) PRO and (b) FLU mixture.

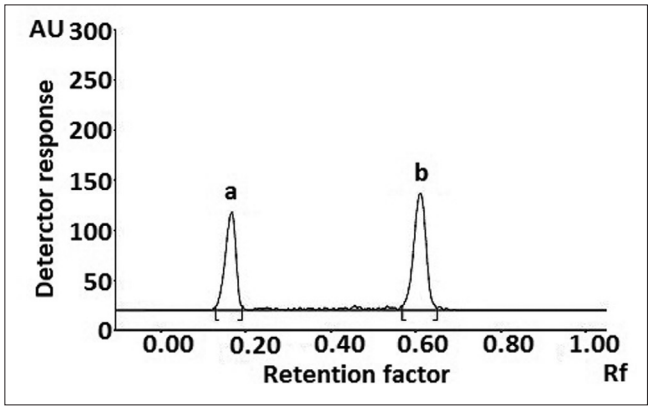

Fig. 3: HPTLC chromatogram of formulation.
HPTLC chromatogram of (a) PRO and (b) FLU mixture.

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