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Stability-indicating high-performance thin-layer chromatographic (HPTLC) method for estimation of Pramipexole dihydrochloride

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Abstract

A simple, selective, precise and Stability-indicating High-performance thin-layer chromatographic method for analysis of Pramipexole dihydrochloride both in a bulk and in pharmaceutical formulation has been developed and validated. The method employed, HPTLC aluminium plates precoated with silica gel as the stationary phase. The solvent system consisted of n-hexane: ethanol: ammonia (3.5: 6: 0.5 v/v). The system was found to give compact spot for Pramipexole dihydrochloride (Rf value of 0.43±0.02). Densitometric analysis of Pramipexole dihydrochloride was carried out in the absorbance mode at 261 nm. The linear regression analysis data for the calibration plots showed good linear relationship with r2 = 0.999±0.0001 with respect to peak area in the concentration range 100 - 600 ng per spot. The method was validated for precision, recovery and robustness. The limits of detection and quantification were 3.57 and 10.82 ng per spot, respectively. Pramipexole dihydrochloride was subjected to acid and alkali hydrolysis, oxidation and thermal degradation. The drug undergoes degradation under acidic, basic, oxidation and thermal conditions. This indicates that the drug is susceptible to acid, base, oxidation and thermal conditions. The degraded product was well resolved from the pure drug with significantly different Rf value. Statistical analysis proves that the method is repeatable, selective and accurate for the estimation of investigated drug. The proposed developed HPTLC method can be applied for identification and quantitative determination of Pramipexole dihydrochloride in bulk drug and pharmaceutical formulation.

Keywords: Pramipexole Dihydrochloride; HPTLC; Validation; Stability; Degradation.

Introduction

Pramipexole dihydrochoride is chemically known as s)-2-amino-4,5,6,7- tetrahydro -6- (propylamino) benzothiazole dihydrochloride. The molecular formula is $C_{10}H_{17}N_3S.2HCl.H_2O$. This corresponds to a molecular weight of 302.26 g/mol. It is used in the treatment of Antiparkinson. It can improves the ability to move and decrease shakiness (tremor), stiffness, slowed movement, and unsteadiness. Pramipexole dihydrochloride non-ergoline dopamine agonist indicated for treating early stage Parkinson's disease (PD) and restlesslegs syndrome (RLS) [1]. It is also sometimes used off-label as a treatment for cluster headache and to counteract the problems with sexual dysfunction experienced by some users of the selective serotonin reuptake inhibitor , (SSRI) antidepressants [2].
Literature survey revealed Several chromatographic methods including liquid chromatography –(LC–) [3-6], validated chiral LC Method enantiomertric seprations [6], and HPLC mass spectrometry (LCMS) [8] have been developed to measure Pramipexole dihydrochloride in biological fluids. However to our knowledge no information related to the stability indicating high performance thin-layer chromatography (HPTLC) determination of Pramipexole dihydrchloride in pharmaceutical dosage forms has ever been mentioned in literature. HPTLC is a widely used analytical technique due to its advantages of low operating cost, high sample throughput, and minimum sample preparation requirement. The major advantage of HPTLC is that several samples can be run simultaneously using a small quantity of mobile phase unlike HPLC, thus reducing the analysis time and cost per analysis [9].

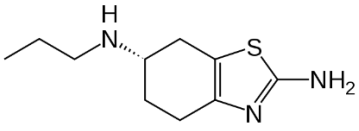

Figure 1. Chemical structure of Pramipexole Dihydrochloride

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Hence, the objective of the present study was to develop a stability-indicating HPTLC method [10] for estimation of Pramipexole dihydrochloride as bulk drug and in formulations and to perform stress studies under a variety of ICH-recommended test conditions [11]. The proposed method was validated for linearity, accuracy (recovery studies), specificity, precision, robustness, ruggedness, LOD (limit of detection), LOQ (limit of quantitation), and repeatability according to the ICH guidelines [10, 11] and its updated international convention .

Experimental

Chemicals and reagents

Pramipexole dihydrochloride was supplied as a gift sample from Torrent pharmaceutical LTD, Mhesana, India. All chemicals and reagents used were of Analytical grade and were purchased from Merck Chemicals, India.

HPTLC instrumentation

The samples were spotted in the form of bands of 6 mm width with a Camag microlitre syringe on precoated silica gel aluminium plates 60 RP-18 F_{254} (10 × 10 cm with 250 mm thickness, E. Merck), using a Camag Linomat 5 applicator. The plates were prewashed with methanol and activated at 60 ^{0}C for 5 min prior to chromatography. The slit dimension was kept at 6.00 × 0.45 mm (micro) and 20 mm/s scanning speed was employed. The mobile phase consisted of N hexane: ethanol: Ammonia (3.5: 6: 0.5 *v*/*v*), and 10 ml of mobile phase was used. Linear ascending development was carried out in a 10 × 10 cm twin trough glass chamber (Camag, Muttenz, Switzerland) saturated with the mobile phase. The optimized chamber saturation time for the mobile phase was 20 min at room temperature (25^{0}C±2). The length of the chromatogram run was approximately 8 cm. Subsequent to development; the TLC plates were dried in a current of air with the help of an air dryer. Densitometric scanning was performed on a Camag TLC scanner 3 and was operated by WINCats software.

Preparation of standard solution and linearity study

An accurately weighed quantity of 5 mg Pramipexole dihydrochloride was transferred to 10 ml volumetric flask, dissolved in methanol and volume was made up to mark with the same solvent to obtain concentration 50 ng/µl. Aliquots of standard solutions 2, 4, 6, 8, 10 and 12µl of Pramipexole dihydrochloride was applied on TLC plate with the help of hamilton syringe, using Linomat 5 sample applicator to obtained the concentration of 100, 200, 300, 400, 500 and 600 ng per spot. The standard curves were evaluated for intra and interday reproducibility. Each experiment was repeated six times.

Method Validation

Precision

Repeatability of sample application and measurement of peak area were carried out using six replicates of the same spot (300 ng per spot of Pramiprexole dihydrochloride). The intra and inter-day variation for the determination of Pramipexole dihydrochloride was carried out at three different concentration levels of 200, 300 and 400 ng per spot.

Limit of detection (LOD) and limit of quantification (LOQ)

In order to determine detection and quantification limit, Pramipexole dihydrochloride concentrations in the lower part of the linear range of the calibration curve were used. Pramipexole dihydrochloride solutions of 100, 120, 140, 160, 180 and 200 ng/spot were prepared and applied in triplicate. The LOQ and LOD were calculated using equation 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.

Specificity

The specificity of the method was ascertained by analyzing standard drug and sample. The spot for Pramipexole dhydrochloride in sample was confirmed by comparing the R_f values and spectra of the spot with that of standard. The peak purity of Pramipexole dihydrochloride was assessed by comparing the spectra at three different levels, i.e., peak start (*S*), peak apex (*M*) and peak end (*E*) positions of the spot.

Ruggedness

Ruggedness of the method was performed by spotting 300 ng of Pramipexole dihydrochloride by two different analyst keeping same experimental and environmental conditions.

Accuracy

The analysed samples were spiked with extra 80, 100 and 120% of the standard Pramipexole dihydrochloride and the mixture were analysed by the proposed method. At each level of the amount, six determinations were performed. This was done to check the recovery of the drug at different levels in the formulations.

Robustness

By introducing small changes in the mobile phase composition, the effects on the results were examined. Mobile phases having different composition of N-hexane-ethanol-Ammonia (3.5:6:0.5 and 4:5.5:0.5, v/v) were tried and chromatograms were run. The amount of mobile phase, temperature and relative humidity was varied in the range of ±5%. The plates were prewashed by methanol and activated at 60 ± 5^0C for 20, 25 and 30 min prior to chromatography. Time from spotting to chromatography and from chromatography to scanning was varied from 0, 20 and 40 min.

Application of proposed method to tablet formulation

To determine the concentration of Pramipexole dihydrocholride in tablets (labeled claim: 25 mg per tablet), the contents of ten tablets were weighed, their mean weight determined and they were finely powdered. The Pramipexole dihydrochloride powder equivalent to (25mg) was weighed. The drug from the powder was extracted with methanol. To ensure complete extraction of the drug, it was sonicated for 20 min and the volume was made up to 100 ml. The resulting solution was filtered using 0.41 µm filter (Millifilter, Milford, MA). The above solution (300 ng per spot) was applied on TLC plate followed by development and scanning. The analysis was repeated in triplicate.

Forced degradation of Pramipexole dihydrochloride

Acid and base induced degradation

The 5 mg of Pramipexole dihydrochloride was separately dissolved in 5 ml of methanolic solution of 50 ml HCl (3N) These solutions were kept for 1 h at room temperature in the dark in order to exclude the possible degradative effect of light. The 10 ml of above solutions was taken and neutralized, then neutralized with NAOH (3 N). That solution neutralized with 5mg Pramipexole dihydrochloride was dissolved water and water and acetonitrile (75:25v/v) The resultant solution were applied on TLC plate in triplicate (4 µl each, i.e. 200 ng per spot) and the chromatograms were run.

Alkaline degradation

5 mg drug was dissolved in the 5 mL of methanol. 50 mL of the 0.5 mol L^{-1} sodium hydroxide was added to it. The solution was kept for 1 h. 10 mL of solution was taken from it and neutralized with 0.5 mol L^{-1} hydrochloric acid. Then the solution was diluted with diluent that is 5mg drug was dissolve in the 5 ml of water to prepare working solution of 5 µg mL^{-1} (pH of solution was 14). (4µL i.e. 200 ng per band) and the chromatograms were run.

Oxidative Degradation

5 mg drug was dissolved in 5 mL of methanol and 50 mL of 30% H_2O_2 was added. The solution was kept for 4 h. Then the solution was diluted with 5 mg drug in 5 ml water to prepare working solution of 5 µg mL^{-1}. (4µL, i.e. 200 ng per band) was applied on a TLC plate and chromatograms were run.

Photo degradation Product

10 mg of drug is exposed to the short wavelength (230 nm) and long wavelength (284 nm) UV light for 48 h. Then the working solution was prepared using diluent A and diluent B. (2µL, i.e. 200 ng per band) of three replicates was obtained.

Results and discussion

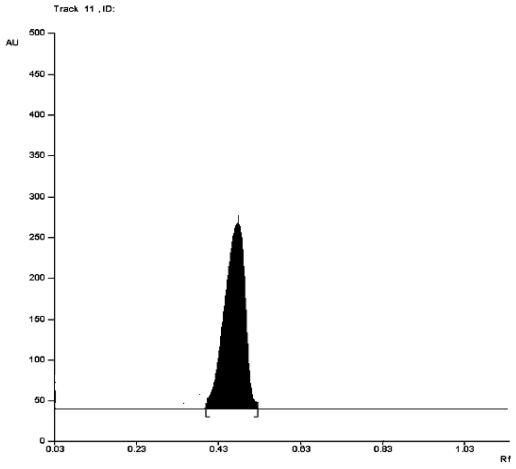

Figure 2. A typical HPTLC chromatogram of Pramipexole Dihydrochloride (RF = 0.43) in N-hexane: ethanol: Ammonia (3.5:6:0.5 v/v) at 261 nmCalibration curve.

Development of optimum mobile phase

TLC procedure was optimized with a view to developing a stability-indicating assay method. Initially, ethylacetate:ethanol: (6:4v/v) gave good resolution with R_f value of 0.4 for Pramipexole dihydrochloride but typical peak nature was missing. Finally, the mobile phase consisting of N-Hexane: Ethanol: Ammonia (5.5:4:0.5*v/v*) gave a sharp and well defined peak at R_f value of 0.43 (Figure 2). Well-defined spots were obtained when the chamber was saturated with the mobile phase for 20 min at room temperature. The linear regression data for the calibration curves showed good linear relationship over the concentration range 100-600 ng/spot.

Table 1. Linear regression data for the calibration curves (n=3).

Linearity range (ng per spot)	100 -600
R^2 ± S.D.	0.999 ± 18
Slope ± S.D	4.585 ± 18
Intercept ± S.D	117.3 ± 18

Validation of method

Precision

The precision of the developed HPTLC method was expressed in terms of % relative standard deviation (% R.S.D.). The results depicted revealed high precision of the method is presented in Table 2.

Table 2: Intra-day and Inter-day precision of HPTLC method (mean of three estimations LOD and LOQ)

Drugs	Conc. ng/spot	Intra-day		Inter-day	
		S.D.	% R.S.D.	S.D.	% R.S.D
Pramipexole Dihydrochloride	200	2.55	0.34	7.1	0.84
	300	6.63	0.52	3.32	0.26
	400	6.28	0.24	6.12	0.23

The LOQ and LOD of 3.57 and 10.82 ng respectively indicates adequate sensitivity of the method.

Recovery studies

The proposed method when used for extraction and subsequent estimation of Pramipexole dihydrochloride from the pharmaceutical dosage form after over spotting with 80, 100 and 120 % of additional drug; afforded good recovery of Pramipexole dihydrochloride The amounts of drug added and determined and the % recovery are listed in Table 3.

Table 3: Recovery study

Drug	Label claim (mg/tablet)	Amount of standard drug added (%)	Drug Recovered	%R.S.D.
Pramipexole Dihydrochloride	0.25	0	100.25	1.15
		80	100.34	1.56
		100	99.15	1.05
		120	100.99	1.73

* mean of three estimations at each level

Specificity

The peak purity of Pramipexole dihydrochloride was assessed by comparing the spectra at peak start, peak apex and peak end positions of the spot, i.e., *r*2 (*S*, *M*) = 0.9998 and *r*2 (*M*, *E*) = 0.9988. Good correlation (r^2 = 0.9989) was also obtained between standard and sample spectra of Pramipexole dihydrochloride (Figure 3).

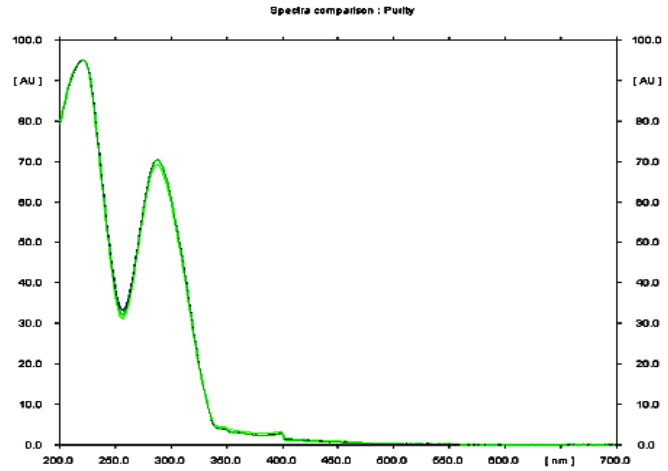

Figure 3. A typical overlay spectrum of standard drug and drug extracted from tablet

Robustness of the method

The standard deviation of peak areas was calculated for each parameter and %R.S.D. was found to be less than 2%. The low values of %R.S.D. values as indicated are shown in Table 4 indicated robustness of the method.

Table 4: Robustness of the method (n=6)

Parameter	S.D. of peak area	% R.S.D.
Mobile phase composition	5.37	0.81
Mobile phase volume	60.44	0.92
Development distance	86.25	1.32
Activation of TLC plate	41.08	0.62
Duration of saturation	85.75	1.36
Time from spotting to chromatography	106.51	1.61
Time from chromatography to scanning	152.54	2.29

Analysis of the marketed formulation

A single spot at R_f 0.43 was observed in the chromatogram of the drug samples extracted from tablets.

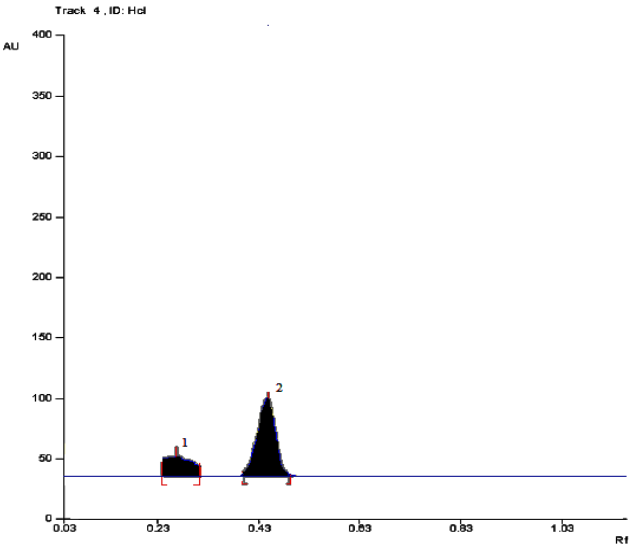

Figure 4a. HPTLC Chromatogram of acid treated Pramipexole Dihydrochloride; peak1 (impurity) (R_f: 0.26), peak2 (Pramipexole Dihydrochloride) (R_f: 0.43).

There was no interference from the excipients commonly present in the tablet. The % drug content and % RSD were calculated. The low % RSD value indicated the suitability of this method for the routine analysis of Pramipexole dihydrochloride in pharmaceutical dosage forms.

Table 5. Summary of validation parameter

Parameter Data	Pramipexole Dihydrochloride
Linearity range (ng per spot)	100 – 600
Correlation coefficient	0.987
Limit of detection (ng per spot)	3.57
Limit of quantification (ng per spot)	10.82
Recovery (*n* = 6)	100.34
Ruggedness(%R.S.D)	
Analyst-I(n=6)	0.65
Analyst-II(n=6)	1.34
Precision (%R.S.D.)	
Repeatability of application (*n* = 6)	0.78 - 0.95
Inter-day (*n* = 6)	0.13 – 0.29
Intra-day (*n* = 6)	0.55 – 0.67
Robustness	Robust
Specificity	Specific

Forced degradation

The chromatogram of the acid degraded samples for showed additional peak at R_f value of 0.25 (Figure 4a), base degraded drug shows at 0.15 (Figure 4b), hydrogen peroxide shows at 0.16 and 0.51 (Figure 4c) respectively. The chromatograms of photo-degraded drugs shows at 0.81 (Figure 4d) and dry heat degraded drug shows at 0.26 and 0.79 (Figure 4e) samples of Naftopidil showed only the spots of the pure drug. The spot of the degraded product was well resolved from the Pramipexole dihydrochloride spot. In both cases, the concentration of the drug was changing from the initial concentration, indicating that Pramipexole dihydrochloride undergoes degradation under acidic, basic and oxidative conditions. The lower R_f values of degraded components indicated that they were less polar than the analyte itself. The results are listed in Table 6.

Table 6. Degradation of Pramipexole Dihydrochloride

Sample exposure condition	No. of degradation product (R_f)	Pramipexole Dihydrochloride remained (ng/600ng) (±SD, n=3)	Recovery (%)
3 M HCl (1h, RT)	1 (0.26)	243.79	81.26
0.5 M NaOH (1h, RT)	1(0.15)	164.65	54.88
30% H_2O_2 (4 h, RT)	2 (0.16,0.51)	106.11	35.37
Photo (48h)	1(0.80)	304.75	101.58

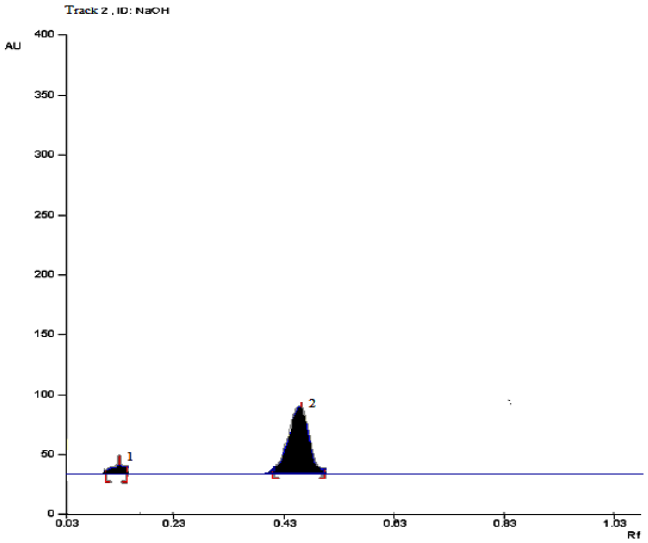

Figure 4b. HPTLC Chromatogram of base treated Pramipexole Dihydrochloride; peak1 (impurity) (R_f: 0.15), peak2 Pramipexole Dihydrochloride (R_f: 0.43)

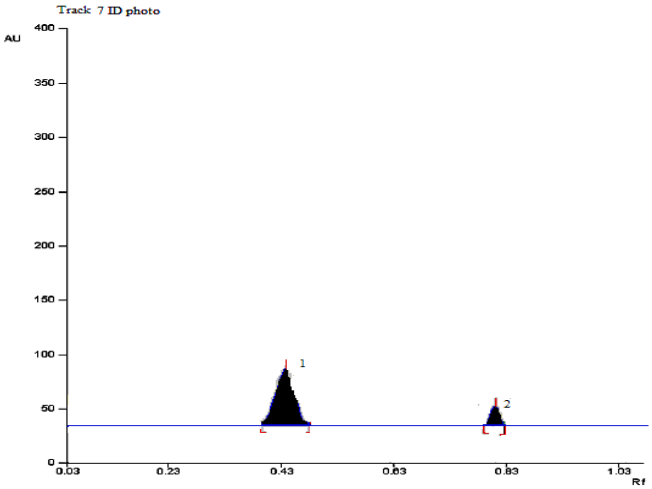

Figure 4c. HPTLC Chromatogram of H_2O_2 treated Pramipexole Dihydrochloride; peak1 (impurity) (R_f: 0.16), peak 3 (impurity) (R_f: 0.51), peak 2 (Pramipexole Dihydrochloride) (R_f: 0.43).

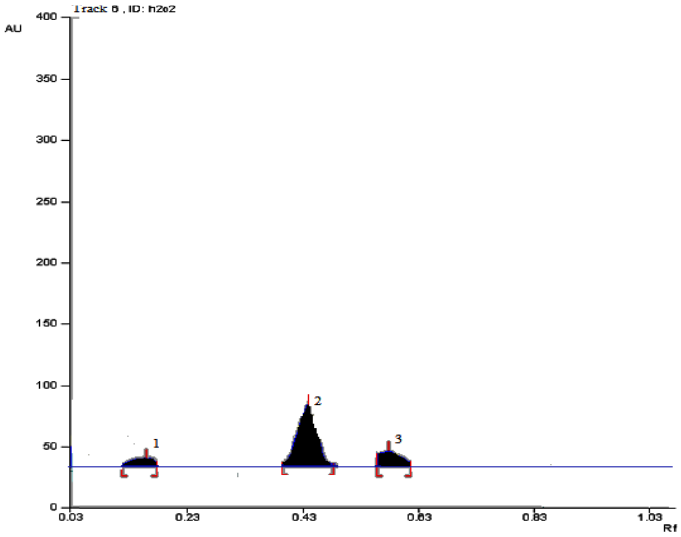

Figure 4d. HPTLC Chromatogram of photo degraded (48 h) treated Pramipexole Dihydrochloride; peak 1 Pramipexole Dihydrochloride (R_f: 0.43), peak 2 (impurity) (R_f: 0.80)

Conclusion

The developed HPTLC method was precise, specific, accurate and stability indicating and validated based on ICH guidelines. Statistical analysis proves that the method is repeatable and selective for the analysis of Pramipexole dihydrochloride as bulk drug and in pharmaceutical formulations. The method can be used to determine the purity of the drug available from the various sources by detecting the related impurities. As the method separates the drug from its degradation products, it can be employed as a stability indicating one.

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