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Validated Stability Indicating HPTLC method for the Quantitative Estimation of Iloperidone in Pharmaceutical Dosage form

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ABSTRACT

Iloperidone is a novel antipsychotic drug widely used to treat schizophrenia. Objective of this investigation was to develop a validated stability indicating high performance thin layer chromatographic method for the quantification of iloperidone in bulk and pharmaceutical dosage form. Aluminium backed TLC plates precoated with silica gel 60F-254 was employed as the stationary phase and n-propanol: chloroform (5:5 v/v) as the mobile phase. Densitometric analysis was performed at 275 nm in the reflectance mode. Compact spots of iloperidone with R_f value 0.36 were observed. Validation of the method as per ICH guidelines produced satisfactory results of linearity (r^2>0.999), limit of detection (5.349 ng/spot), limit of quantification (16.2099 ng/spot), precision (< 2%), and accuracy (99.66 ±0.341 to 100.34±0.292%). Degradation products were found to be well separated from the pure drug with significantly different R_f values suggesting a stability indicating analysis method for the estimation of iloperidone in pharmaceutical preparations and as bulk drug. The proposed method is selective, sensitive, precise, and accurate. It is also simple, economic and time saving as compared to reported HPLC methods.

Keywords: Iloperidone, stability indicating, HPTLC, validation, ICH guidelines, degradation

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INTRODUCTION

Iloperidone is an antipsychotic drug used in the treatment of schizophrenia. Chemically it is designated as 1-[4-[3-[4-(6-fluoro-1,2-benzisoxazol-3-yl)-1-piperidinyl]propoxy]-3-methoxyphenyl] ethanone with a molecular formula $C_{24}H_{27}FN_2O_4$ and molecular weight 426.48[1]. The parent drug stability test guidelines (Q_1A) issued by the International Conference on Harmonisation (ICH) requires that analytical test procedures for stability samples should be fully validated and the assays should be stability-indicating[2,3]. A thorough literature survey reveals that validated assays using derivative spectrophotometric methods[4], stability indicating HPLC method in bulk and pharmaceutical dosage forms[5] and LC-MS method for quantification in plasma[6] have been reported for iloperidone but there is no stability indicating HPTLC method for its quantification in formulations. A notable advantage of HPTLC is that several samples can be run simultaneously using a relatively small quantity of mobile phase, thus lowering analysis time and cost. Technically it is very simple to operate, facilitates automated sample application and scanning in situ. It offers extreme flexibility for various components such as mobile phase, stationary phase, developing techniques and detection (pre and post chromatographic determination). It also permits repeated detection of the densitogram using same or different parameters[3]. The purpose of the present work was to practice the ICH recommendations by subjecting iloperidone to various stress conditions like, hydrolysis, oxidation, photolysis and thermal degradation to establish its inherent stability characteristics, and to develop a validated stability indicating HPTLC assay method for the estimation of iloperidone in bulk and pharmaceutical formulation.

MATERIALS AND METHODS

Reference sample of iloperidone (assigned purity > 99% w/w) was used for the study. All chemicals and reagents used were of analytical grade and were procured from Merck Chemicals, India. High-purity water prepared using Millipore purification system (Milli-Q, Bangalore, India) was used throughout the analysis.

Instrumentation

Samples were spotted as bands (6 mm wide and 6 mm apart) by means of a CAMAG (Muttenz, Switzerland) Linomat 5 sample applicator equipped with a 100 µL syringe (Hamilton, Bonaduz, Switzerland) on a 20x10 cm aluminum sheet pre-coated with silica gel $60F_{254}$ of 250 µm thickness (E. Merck, Darmstadt, Germany). The plates were prewashed with methanol and activated at 110°C for 5 minutes prior to chromatographic analysis. The slit dimension was

5×0.45 mm and the scanning speed was 20 mm/Sec. Linear ascending development was performed using a twin-trough glass chamber of 20x10 cm (Model 022.5253, CAMAG). Densitometric scanning was done using CAMAG TLC Scanner 3 at 275 nm in the reflectance mode. The scanner was controlled by winCATS software (Version 1.2.6). The radiation source used was the deuterium lamp which emits a continuous UV spectrum between 200-400 nm.

Preparation of standard solutions

Stock solution was prepared by dissolving 2 mg of iloperidone in 10 ml methanol and the solution was made up to 25 ml. From this 160, 200, 240, 280, 320 ng/spot were obtained by spotting 2, 2.5, 3, 3.5, and 4 µL on TLC plates.

Optimization of variants in TLC and fixing of initial chromatographic conditions

Effect of experimental variables such as mobile phase composition, chamber saturation time, plate equilibration time, band width of the spot and solvent front on the R_f value of the drug were evaluated. Initially, various mobile phase ratio and compositions were tried to produce compact spot for iloperidone. Solubility of iloperidone and polarity of solvent system were taken into consideration in this stage. Following the development of chromatogram, the bands were scanned in the range of 200-400nm. The wavelength at which maximum absorbance obtained was selected as the detection wavelength. Chamber saturation times of 10 to 20 minutes were tried. Plate equilibration time also has a major effect in obtaining reproducible results in HPTLC. The mobile phase was taken in one side of a twin trough chamber and spotted plates on the other side. The plates were kept for equilibration with the vapour phase for 25-30 minutes. In order to determine the effect of solvent front, the plates were developed with a distance of solvent front ranging from 7.0 to 9.0 cm. Band width was optimized through several trials in order to obtain good peak shape and reproducibility of R_f values. The effect was also studied at lowest and highest volume spotted (2-4 µl). After development, densitometric evaluations were carried out in order to understand the effect of the above mentioned variables over the peak shape and R_f values of iloperidone and thus to fix the initial chromatographic conditions.

Validation of the method

The optimized HPTLC method was validated in compliance with ICH guidelines in terms of the following parameters[7].

Specificity

The specificity of the developed method was evaluated by comparing the R_f values and spectrum of the solutions of standard and test containing excipients normally used in the formulation.

Linearity and range

Linear relationship between peak area and concentration was evaluated over the concentration range expressed in ng/spot by making six measurements at five concentration levels in the range of 160-320 ng/spot. Calibration curves were generated by plotting the peak area against the corresponding concentrations.

Precision

The precision of the method was verified by repeatability and intermediate precision studies. Repeatability studies were performed by replicate analyses(n=6) of three different concentrations (160, 240 & 320ng/spot) of the drug in six times on the same day. The intermediate precision of the method was confirmed by repeating the study on three different days.

Accuracy

Recovery studies were carried out by spiking the pre-analyzed samples of iloperidone with known concentrations of standards at two different levels (50, 100%) and the samples were analyzed by the described method.

Limit of detection and limit of quantification

The LOD and LOQ were determined using the calibration curve.

LOD = 3.3xσ/S and

LOQ = 10 x σ/S,

where, σ = standard deviation of y intercept of regression line

S = slope of the calibration curve

Robustness of the method

The results were noted after introducing small deliberate variations in the mobile phase composition, its volume, chamber saturation time and solvent migration distance. Mobile phase having differing compositions of n-propanol and $CHCl_3$ (4.9:5.1 and 5.1:4.9 v/v) were tried. Mobile phase volume and duration of chamber saturation were varied at 20±2ml and 20±2min respectively. The experiment was performed in six replicates at a concentration level of 200 ng/spot and the %RSD of peak area was calculated.

Stability Studies

Solution stability studies were performed under room temperature for 24 hours and under refrigeration for 5 days.

Analysis of a marketed formulation

To determine the content of iloperidone from tablet dosage (label claim: 2 mg), twenty tablets were weighed; their mean weight was determined and finely powdered. The weight of the tablet

triturate equivalent to 2 mg of iloperidone was transferred into a 25 ml volumetric flask containing methanol, sonicated for 30 minutes for complete extraction of the drug, filtered using whatman filter paper (no.1). Then 2 µL was spotted (160 ng/spot) on TLC plate, developed and evaluated densitometrically as described above. The analysis was repeated in six replicates. The possibility of excipient interference with the analysis was also examined.

Forced Degradation Studies

Four samples were used in the experiment, viz., the blank solution stored under normal condition, the blank subjected to stress in the same manner as the drug solution, zero time sample containing the drug which was stored under normal conditions and the drug solution subjected to stress treatment. The study was conducted separately for iloperidone in bulk followed by the formulation containing 2 mg of iloperidone.

To 10 ml methanolic solution of the drug (2 mg), 10 ml of 1N HCL was added, the volume was made upto 25 ml with methanol and refluxed at 70ºC for 3 hours. Similar procedure was followed for alkaline hydrolysis at 80ºC in 1 N NaOH for 5 hours. Then 3µL (320 ng/spot) of the resultant solutions were spotted on TLC plates, developed and analyzed by the above mentioned method. To study the degradation behaviour of iloperidone under neutral conditions, 2 mg of drug was dissolved in methanol, made upto 25 ml using distilled water and refluxed at 80ºC for 8 hours. For oxidative degradation, methanolic solution of the drug was treated with 3% hydrogen peroxide for 5 hrs. The standard drug in solid form was exposed to controlled temperature in oven at 80ºC for 8 days to study the thermal degradation behaviour. Photolytic degradation was performed by directly exposing the methanolic solution of drug to sunlight for 2 days. Average peak areas of six replicate applications were documented.

RESULTSAND DISCUSSION

Optimization of the stability-indicating HPTLC method

HPTLC is widely employed in stability studies because of simplicity, rapidity, economy and overall versatility in quality control aspects of drugs[8]. Mobile phase for the study was selected by considering the sensitivity of the assay, the time needed for analysis, and the use of readily available solvents. Several trials were made with different solvent systems to achieve satisfactory separation between analyte and degradants. Various ratio of chloroform:methanol, chloroform:ethanol and chloroform:ethyl acetate were tried but no spots were seen. Subsequently, the optimized mobile phase for the stability indicating method of iloperidone was composed of n-propanol and chloroform (5:5 v/v). Adequate chamber saturation for 20 minutes

ensured reproducible results and eliminated secondary solvent fronts. The optimized development distance was 8.0 cm with an approximate development time of 25 minutes. For each chromatographic run 20 ml of mobile phase was used. Following development, plates were air dried and densitometric scanning and evaluation was performed at 275 nm.

Validation of the method

The results of validation of the stability indicating method of iloperidone are described below.

Specificity

There were no interferences due to degradants or excipients, indicating the specificity of the method.

Linearity

Good linear relationship was observed over the concentration range 160 to 320 ng/spot (n=5). Correlation coefficients (r^2), *y*-intercepts and slopes of regression line were calculated and presented in table 1. The linear regression equation obtained was 7.881x+341.20.

Table 1:Linear regression data for the calibration curves[a]

Parameters	Values
Linearity range (ng per spot)	160-320
r^2±SD	0.9994±0.00026
Slope±SD	7.984±0.091
Confidence limit of slope	7.758-8.210
Intercept	336.213±12.942
Confidence limit of intercept	304.06-368.37

[a] n = 6, [b] 95% confidence limit.

Accuracy

The % recovery of the drug ranged between 99.66±0.341 to 100.34±0.292%, when used for the estimation of iloperidone after spiking with 50 and 100% of reference standards. Results of recovery studies are shown in table 2. The %RSD of recovery was less than 2.

Table 2: Recovery studies

Conc. of drug taken (ng/spot)	Conc. of standard added (ng/spot)	Mean Conc±SD[a]	Mean Recovery (%) ±SD	%RSD	S.E.[b]
160	80	239.463±0.545	99.6643±0.341	0.3418	0.1976
160	160	320.54±0.4681	100.3374±0.292	0.2914	0.1688

[a] n=6, [b] Standard error.

Precision

Table 3 summarizes the results of repeatability and intermediate precision experiments. The method was found to be precise, reliable and reproducible as the RSD values for repeatability

and intermediate precision studies were within the acceptable range.

Table 3:Intra- and Inter-day precision of HPTLC method[a]

Amount (ng/spot)	Intra-day Precision			Inter-day Precision		
	Mean conc ±SD	%RSD	S.E.[b]	Mean conc ±SD	RSD	S.E.[b]
160	160.14±0.401	0.250	0.1635	159.843±0.853	0.534	0.3483
240	240.3958±0.705	0.293	0.2877	240.444±0.550	0.229	0.2244
320	320.3783±0.512	0.160	0.2088	320.6033±0.580	0.181	0.2367

[a] n=6, [b] Standard error

LOD and LOQ

The LOD and LOQ estimated by the standard deviation method were 5.349 and 16.209 ng/spot respectively, signifying the sensitivity of the developed method.

Robustness of the method

The standard deviations of peak areas were calculated for the aforementioned four parameters (mobile phase composition, mobile phase volume, chamber saturation time and solvent migration distance) at a concentration level of 200 ng/spot and coefficients of variation were found to be less than 2% in all the cases as shown in table 4. The low CV values indicate the robustness of the method.

Table 4: Robustness of the method[a]

Parameter	S.D of peak area	%RSD
Mobile Phase composition	0.8963	0.046
Mobile phase volume (ml)	1.607	0.0831
Chamber saturation time (min)	1.601	0.0828
Solvent migration distance (mm)	1.872	0.0968

[a] n=6

Analysis of marketed formulation

The extracted samples containing iloperidone showed a single spot at R_f 0.36. Excipients present in the formulation did not interfere with the peak. The percentage amount of iloperidone present in the tablet formulation was 99.756±0.670. The low % RSD values indicate the suitability of the method for the routine quality control of iloperidone in tablet dosage forms. The results of assay are shown in table 5.

Table 5: Assay of iloperidone in formulation by HPTLC Method

Drug	Labeled amount (mg/tab)	Amount found (mg)±SD[a]	Assay (%)	RSD[a]
Iloperidone	2	1.9951±0.0134	99.756	0.6716

[a]n=6

Forced degradation studies

Iloperidone showed 17.65% and 23.84% degradation under the given conditions of acid and

alkaline hydrolysis respectively.

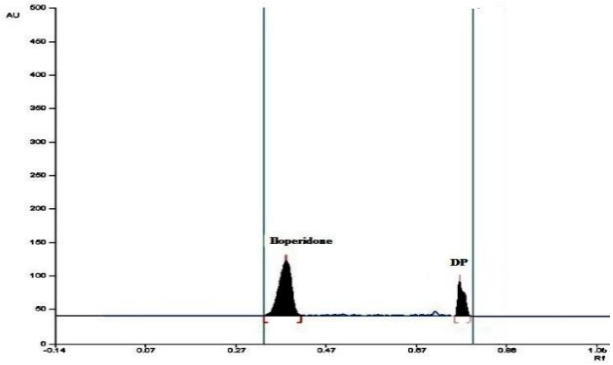

Figure 1: Densitogram of acid treated iloperidone (1 N HCl -reflux at 70°C for 3 hours)

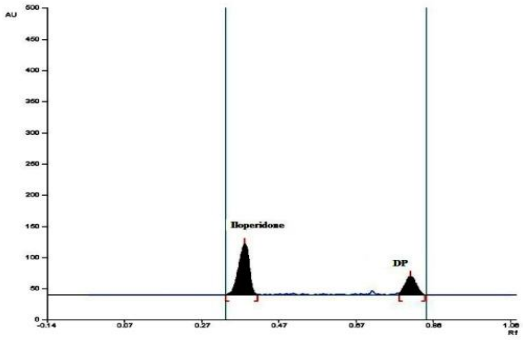

Figure 2: Densitogram of base treated iloperidone (1 N NaoH - reflux at 80°C for 5 hours)

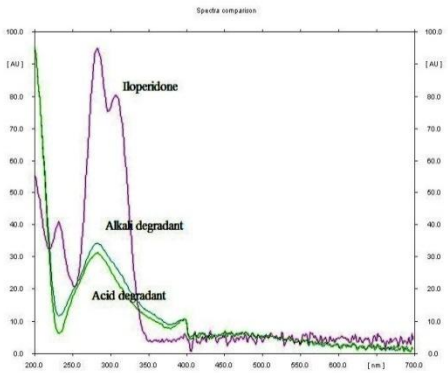

Figure 3: Overlay spectra of iloperidone, acid and alkali induced degradation products.

The densitogram of acid and alkaline hydrolysis of iloperidone are shown in figure 1 and 2. Figure 3 shows the overlay spectrum of the drug and degradation products. The drug was found to be stable to neutral hydrolysis, oxidation, photolysis and thermal degradation where no major degradation products were found. Table 6 presents the results of stress studies. It is evident that the analyte spots (R_f 0.36) were well resolved from degradation products of acid (R_f 0.8) and alkali (R_f 0.81) hydrolysis. The product formed from acid degradation may be similar to that of base induced degradation as indicated by both R_f values and spectral characteristics. Under mild acidic conditions (0.01 N HCL), degradation was extremely slow even after 8 hours of reflux

while 17.65% degradation was observed after 3 hours in 1N HCl. The findings of the present study are in agreement with that of stability indicating HPLC method by Mandpe, 2011[5] who has reported significant degradation of iloperidone in 0.1N HCl after 8 hours and 1N NaOH after 2 hours of reflux at 80ºC.The results obtained clearly indicate that the drug is very stable and undergoes degradation only under extreme conditions of stress. The single spot at R_f value 0.36 in the sample from tablet formulation clearly shows that no degradation of iloperidone occurred in the formulation. Assay result obtained from the proposed method was compared with reported methods[5,9] using one way ANOVA (F-test) at $p<0.05$. F value was found to be 1.107, which is less than the tabulated F value 3.682. The p value was found to be 0.356. The test ascertains that there is no statistically significant difference between the proposed method and reported methods[5,9].

Table 6: Summary of degradation studies of iloperidone

Stress condition	%Drug remained	R_f	
		Drug	DP*
1 N HCl (reflux at 70°C for 3 hours)	82.3440	0.36	0.80
1 N NaoH (reflux at 80°C for 5 hours)	76.1559	0.36	0.81
Neutral (Reflux at 80°C for 8 hours)	99.14	0.36	-
Oxidative degradation (3% hydrogen peroxide for 5 hrs)	96.65	0.36	-
Dry heat (80°C for 8 days)	99.82	0.36	-
Photolysis (direct sunlight for 2 days)	99.62	0.36	-

DP* degradation product

Stability studies

Sample solutions were found to be stable upto 24 hours in room temperature and upto 5 days under refrigeration.

CONCLUSION

The developed HPTLC technique was found to be simple, rapid, selective, sensitive and stability-indicating. The degradants were well resolved from the analyte peak. Statistical analysis proved that the method is precise and accurate. The developed method can be used for the routine quality control tests of pharmaceutical dosage forms and stability samples. The method can minimize the cost of reagents and time of analysis. Thus, it can represent another good alternative for the already existing stability indicating HPLC method, especially those using certain type of detectors which are not present in most of the laboratories.

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