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Stress degradation studies on Iloperidone and development of a stability-indicating HPLC method for bulk drug and pharmaceutical dosage form

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ABSTRACT

A very sensitive stability indicating RP-HPLC method has been developed and validated for iloperidone in the presence of its degradation products generated in studies of stressed decomposition. Iloperidone is recently approved and marketed in US for the treatment of psychosis. This method is based on HPLC determination of iloperidone by using C-18 column (250 mm × 4.6 mm, 5.0 μ) with isocratic conditions and simple mobile phase containing acetonitrile: 0.025M KH_2PO_4: triethylamine (60:39.9:0.1) at flow rate of 1 mL/min using UV detection at 229 nm. This method has been applied to formulation without interference of excipients of formulation and was validated with respect to linearity, precision, accuracy, and selectivity. The linear regression analysis data for the calibration plots showed a good linear relationship over the concentration range of 100-600 ng/mL. The mean values of the correlation coefficient, slope and intercept were 0.9987, 0.00047 and 5.789149, respectively for iloperidone. The limit of detection (LOD) and limit of quantitation (LOQ) was 30 ng/mL and 60 ng/ml respectively. Statistical analysis showed that the method is repeatable and selective for the estimation of iloperidone as the bulk drug and in pharmaceutical preparations. The drug substances were subjected to stress by acid and base hydrolysis (0.1 N HCl and 1 N NaOH), oxidation (6% H_2O_2), neutral degradation, photolysis, and dry heat degradation studies (80°C). The drug was degraded under acidic, basic and oxidative conditions but was stable under other stress conditions investigated. Because the method effectively separates the drug from its degradation products, it can be used as stability-indicating.

Keywords: Iloperidone, HPLC, validation, stability indicating, stress degradation.

INTRODUCTION

Iloperidone, (1-[4-[3-[4-(6-fluoro-1,2-benzisoxazol-3-yl)-1-piperidinyl]propoxy]-3-methoxy phenyl]ethanone) (Figure 1) is a second generation atypical antipsychotic agent recently approved by United States Food and Drug Administration and is indicated for the acute treatment of schizophrenia in adults. Iloperidone is a white to off-white finely crystalline powder. It is

practically insoluble in water, very slightly soluble in 0.1 N HCl and freely soluble in chloroform, ethanol, methanol, and acetonitrile. It is commercially available in the form of oral tablets in seven different strengths viz. 1mg, 2mg, 4mg, 6mg, 8mg, 10mg and 12mg. [1, 2].

In the literature, a liquid chromatographic-mass spectrometric (LC-MS) method has been developed by Mutlib et al to quantitate iloperidone, and its principal metabolite, 4-[3-[4-(6-fluoro-l,2-benzisoxazol-3-yl)-l-piperidinyl]propoxy]-3-methoxy-α-methylbenzenemethanol, in human plasm [3]. Sainati et al have reported preliminary pharmacokinetic assessment by HPLC method with ultraviolet detection to support human pharmacokinetic studies based upon a 3 and 5 mg dosage regimen [4].

Literature survey reveals that, no stability indicating analytical method for determination of iloperidone in dosage forms has been published. The objective of this study was, therefore, to develop a new, simple, rapid, precise, accurate, and specific stability-indicating HPLC method through stress studies as per ICH recommended stress conditions [5, 6, 7].

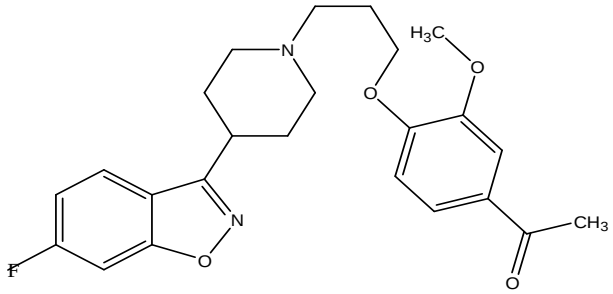

Figure 1 Structure of Iloperidone

MATERIALS AND METHODS

Materials and Chemicals

Iloperidone was kindly provided as a gift sample by Symed Labs, Hyderabad, India. All other chemicals and reagents used were of HPLC grade and purchased from Merck Chemicals, India. Nylon filter paper of 0.45µm (Millipore) was purchased from Pall life science, Mumbai, India. For all analysis, double-distilled water filtered through a 0.45 µm membrane filter was used.

Instrumentation

The HPLC system consisting of an Intelligent LC Pump (model Jasco PU 2080) with autosampler programmed at 20 µL capacity per injection was used. The detector consisted of UV/VIS (Jasco UV 2075) model operated at a wavelength of 229 nm. Data was integrated using JascoBorwin version 1.5, LC-Net II/ADC system. A Thermo Scientific ODS Hypersil (250mm x 4.6mm, 5µ) C-18, column was used for separations. Different mobile phases were tested in order to find the best conditions for eluting the drug. The mobile phase contained a mixture of acetonitrile, buffer and triethylamine in the ratio of 60:39.9:0.1 (v/v) at the flow rate of 1ml/min. The buffer consisted of 0.025 mM potassium dihydrogen phosphate monohydrate, pH adjusted to 6.0 using concentrated aqueous solution of sodium hydroxide.

Iloperidone standard stock solutions

The stock solution of iloperidone was prepared by weighing 10 mg of iloperidone, transferring it into a 10 mL volumetric flask, dissolving and diluting to volume with 10ml methanol to achieve the concentration of 1 mg/mL. This stock solution was stable for at least three months when

stored in refrigerator at 4°C. Working standard solutions of iloperidone were prepared daily by diluting the stock solution to an appropriate concentration with mobile phase.

Optimization of HPLC method

The HPLC procedure was optimized with a view to develop a simple assay method for iloperidone. For optimization of HPLC method, different ratios of methanol:buffer and acetonitrile:buffer with or without triethylamine were tried. 20µl of the standard solution (10 µg/ml) was injected in HPLC at the flow rate of 1 ml/min and using UV detection at 229nm. To get good resolution, the method was further optimized by changing the ratio of acetonitrile, buffer and triethylamine and it was found that acetonitrile:buffer (0.025M KH_2PO_4):triethylamine in the ratio 60:39.9:0.1 v/v, at flow rate of 1 mL/min provided acceptable retention time, peak shape symmetry, plates and good resolution for iloperidone (Figure 2).

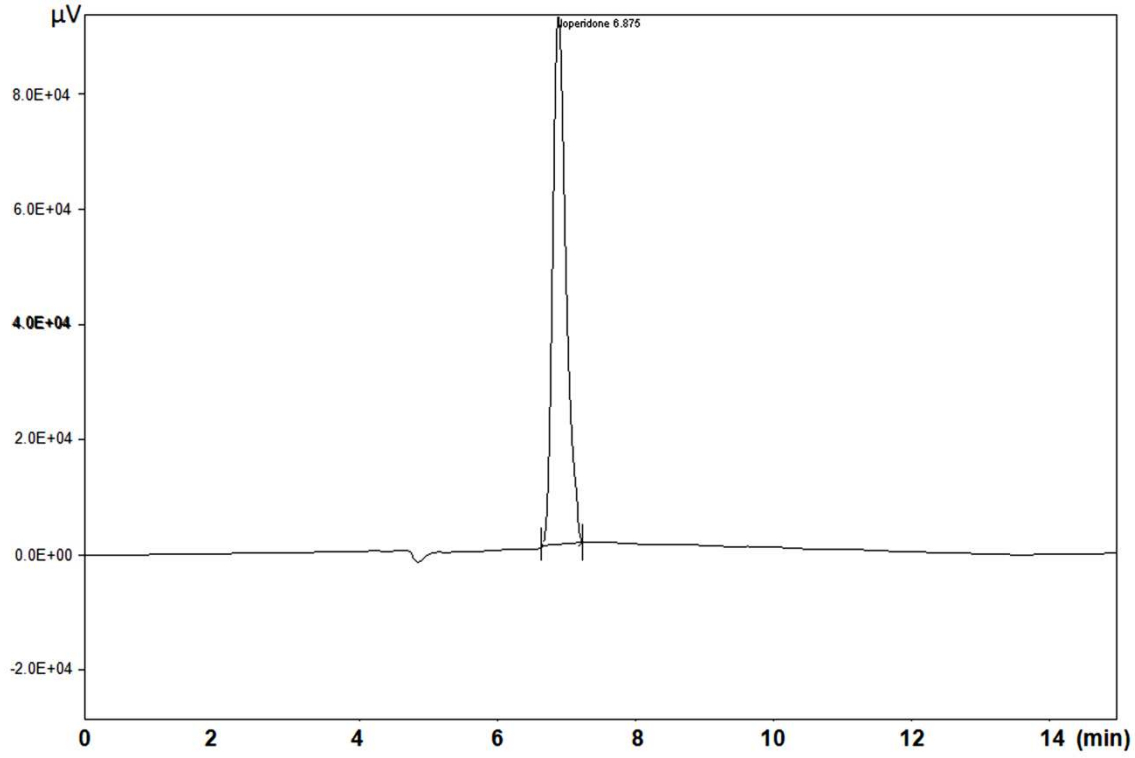

Figure 2 HPLC chromatogram of standard iloperidone (10 µg/mL)

Validation of the method

Validation of the optimized HPLC method was carried out with respect to the following parameters [8, 9, 10].

Linearity and range

The standard stock solution (1000 µg/mL) was further diluted with mobile phase to get set of standard solutions in the concentration range of 100-600 ng/mL. Linearity of the method was studied by injecting these six concentrations of the drug in triplicate into the HPLC system. The peak area was recorded for all the peaks and calibration graph was obtained by plotting peak area ratio versus concentration of iloperidone.

Precision

The precision of the method was determined by repeatability (intra-day) and intermediate precision (inter-day). Repeatability studies were performed by injecting three different

concentrations 200, 400 and 600 ng/mL of iloperidone standard solution three times on the same day and recording the corresponding peak areas. Intermediate precision studies were performed by analysis of three different concentrations 200, 400 and 600 ng/mL three times on three different days. % Relative standard deviation (% RSD) was calculated.

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

The LOD is the smallest concentration of the analyte that gives a measurable response (signal to noise ratio of 3). The LOQ is the smallest concentration of the analyte, which gives response that can be accurately quantified (signal to noise ratio of 10). To determine the LOD and LOQ, serial dilutions of very low concentrations of standard solution of iloperidone were made from the standard stock solution. The samples were injected in HPLC system and measured signal from the samples was compared with those of blank samples.

Robustness of the method

Robustness of the method was determined by making slight changes in the chromatographic conditions, such as change in flow rate, percentage of acetonitrile in the mobile phase. Robustness of the method was done at three different concentration levels viz. 200, 400 and 600 ng/mL for iloperidone.

Specificity

The specificity of the method towards the drug was established through study of resolution factor of the drug peak from the nearest resolving peak. The peak purity of iloperidone was determined by comparing the spectrum at three different regions of the spot i.e. peak start (S), peak apex (M) and peak end (E). Effect of excipients of formulation was studied for its interference with the assay.

Accuracy

To study accuracy of the method, recovery experiment was carried out by spiked concentrations. A known quantity of pure drug substance corresponding to 80, 100 and 120% of label claim was spiked into preanalyzed sample formulation (iloperidone tablet-Fanapt®) against 100% working standard (standard addition method), mixed and the powder was extracted and analyzed by running chromatogram in optimized mobile phase.

Analysis of a marketed formulation

To determine the content of iloperidone in conventional tablet (Brand name: Fanapt®; Label claim: 12 mg), twenty tablets were weighed, their mean weight determined and finely powdered. The weight of the tablet triturate equivalent to 12 mg was transferred into a 50 mL volumetric flask containing 20 mL methanol, sonicated for 30 min and diluted upto 50 mL with methanol. The resulting solution was centrifuged at 3000 rpm for 5 min and the drug content of the supernatant was determined. Supernatant was taken and after suitable dilution the sample solution was then filtered using 0.45-micron filter (Millipore, Milford, MA). The above stock solution was further diluted to get sample solution of 100 and 200 ng/mL for iloperidone. A 20 µl volume of sample solution was injected into HPLC, six times, under the conditions described above. The peak areas were measured at 229 nm and concentrations in the samples were determined using multilevel calibration developed on the same HPLC system under the same conditions using linear regression equation.

Forced Degradation of iloperidone

To determine whether the analytical method and assay were stability-indicating, iloperidone bulk powder was stressed under different conditions in forced degradation studies [5, 6]. For all the

stability study, the formation of degradable product was confirmed by comparing the chromatogram of the degradable mixture with the blank solvent stored under normal condition and the drug solution kept under normal condition.

Acid and Base induced hydrolysis

Hydrochloric acid (HCl) (0.1N, 10 mL) and sodium hydroxide (NaOH) (1N, 10 mL) were separately added to 10 mL methanolic stock solutions of iloperidone (1 mg/ml). These mixtures were separately heated under reflux for upto 8 h at 80°C. The solutions (1 mL) were then withdrawn and transferred to 10 mL volumetric flasks, neutralized by addition of 1N NaOH or 0.1 N HCl, and diluted to volume with mobile phase.

Oxidation

Hydrogen peroxide (H_2O_2; 6%, v/v, 10 mL) was added to 10 mL methanolic stock solutions of iloperidone (1 mg/ml). These solutions were separately heated in a boiling water bath for 10 min to completely remove excess hydrogen peroxide and then set aside for 8 h at ambient temperature in the dark. The solutions (1 mL) were withdrawn at different time intervals, transferred to 10 mL volumetric flasks and diluted to volume with mobile phase.

Neutral Degradation

Aqueous stock solution of iloperidone was heated under reflux for 48 h at 80°C to study the effect of neutral stress. The solutions (1 mL) obtained were transferred to 10 mL volumetric flasks and diluted to volume with mobile phase.

Photo stability

Photo degradation studies were carried out according to option 2 of Q1B in ICH guidelines [7]. Samples were exposed to light for an overall illumination of 1.2 million lux hours and an integrated near ultraviolet energy of 200 watt hm^2. The solutions (1 mL) obtained were transferred to 10 mL volumetric flasks and diluted to volume with mobile phase.

Dry heat degradation

For dry heat degradation study, samples of drug substance were placed in controlled temperature oven at 80°C for 8days. Samples were withdrawn at appropriate time and subjected to HPLC analysis after suitable dilution (0.1 mg/mL).

Results and Discussion

The developed method has many advantages, for example isocratic conditions rather than gradient ion-pairing RP-LC which requires more sophisticated instrumentation and long stabilization times.

The results of validation studies of the method developed for iloperidone in the current study involving acetonitrile: buffer: triethylamine (60:39.9:0.1, v/v) are given in table 1 and 2.

Table 1 Optimized Chromatographic Conditions

Parameter	Optimized Condition
Chromatograph	HPLC (Jasco PU 2080), Intelligent LC pump with programmed sampler
Column	Thermo Hypersil ODS–C18 (250 mm × 4.6 mm, 5.0 µ)
Mobile Phase	Acetonitrile:0.025M KH_2PO_4 (pH 6.0):Triethylamine (60:39.9:0.1 V/V)
Flow Rate	1 ml/min
Detection	229 nm
Injection Volume	20µl
Column Temperature	Ambient

Table 2 System Suitability Parameters

Sr. No.	Parameters	Results Obtained
1	Calibration Range	100-600 (ng/ml)
2	Theoretical Plates	9695
3	Asymmetry	1.129
4	Retention Time	6.875min

Linearity

Iloperidone showed good correlation coefficient (r^2 = 0.9987) in given concentration range (100-600 ng/ml). The calibration curve parameters of iloperidone showed a linear relationship between peak area and concentration. The mean values of the slope and intercept were 0.00047 and 5.7891 for iloperidone.

Precision

The results of the repeatability and intermediate precision experiments are shown in Table 3 and 4. The RSD values for repeatability and intermediate precision studies were < 2 %, as recommended by ICH guidelines thus confirming good precision of the method.

Table 3 Intraday Precision

Concentration (ng/ml)	Area	% RSD
200	405816.5	1.72
	405815.9	
	405817.3	
400	841712.0	1.18
	841711.0	
	841713.0	
600	1324532.0	1.96
	1324528.0	
	1324533.0	

Table 4 Interday Precision

Concentration (ng/ml)	Day	Area	% RSD
200	1	405816.5	1.23
	2	405816.8	
	3	405817.5	
400	1	841712.0	1.78
	2	841711.0	
	3	841714.0	
600	1	1324532.0	1.14
	2	1324529.0	
	3	1324531.0	

LOD and LOQ

Signal-to-noise ratios of 3:1 and 10:1 were obtained for the LOD and LOQ respectively. The LOD and LOQ were found to be 30 ng/mL and 60 ng/mL respectively for iloperidone. From this, it was concluded that the developed method is sensitive.

Robustness of the method

Each factor selected was changed at three levels (−1, 0 and 1). One factor at the time was changed to estimate the effect. Thus, replicate injections (n = 6) of mixed standard solution at three concentration levels were performed under small changes of three chromatographic

parameters (factors). It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP-HPLC method developed is robust. The robustness limit for flow rate variation and mobile phase variation are well within the limit, which shows that the method is having good system suitability and precision under given set of conditions and were within the acceptance criteria of not more than 2%.

Table 5 Robustness testing (n=3)

Factor	Level	Retention Time	Asymmetry
A: Flow Rate			
0.9	-1	6.905	1.131
1.0	0	6.875	1.129
1.1	+1	6.723	1.123
Mean ± SD		6.834 ± 0.09	1.127 ± 0.004
B: % of Acetonitrile in the mobile phase			
58	-1	6.701	1.121
60	0	6.875	1.129
62	+1	6.989	1.119
Mean ± SD		6.855 ± 0.14	1.123 ± 0.005

Specificity

The peak purity of iloperidone was assessed by comparing their respective spectra at the peak start, apex and peak end positions i.e., r (S, M) = 0.9988 and r (M, E) = 0.9988. A good correlation (r = 0.9997) was also obtained between the standard and sample spectra of iloperidone. Also, excipients from formulation were not interfering with the assay.

Recovery studies

Percentage recovery of iloperidone in bulk drug samples was ranged from 99.0 to 100.0 %. The excellent recovery obtained suggests the accuracy of the method is good (Table 6).

Table 6 Accuracy/Recovery Studies

Label Claim (mg/tablet)	Amount Added (mg)	Total Amount (mg)	Amount Recovered ± % RSD	% Recovery
12	9.6 (80 %)	21.6	21.4 ± 0.96	99.07
12	12 (100 %)	24	23.9 ± 1.11	99.58
12	14.4 (120 %)	26.4	26.3 ± 0.75	99.62

Degradation Studies

The chromatograms obtained from samples treated with acid, base and hydrogen peroxide contained well separated peaks of pure iloperidone and additional peaks of their degradation products at different retention times.

The degradation of drug substance was observed only under acidic, basic and oxidative conditions. It was observed that the drug degraded slowly in strongly acidic conditions over a period of time. On reflux in 0.01 N HCl for 12 h there was no degradation observed, however the degradation of the drug in 0.1 N HCl resulted in the rise of one additional peak at 4.983 min after 3 h. This indicates that the drug is hydrolysed under acidic conditions, to a chromatographic compound. (Figure 3)

For alkali degradation in 1 N NaOH, the drug was found to decompose almost 12-15% after refluxing for 2 h resulting in the rise of one additional peak at 4.892 min. (Figure 4)

The drug substance iloperidone under oxidative condition leads to the formation of a unknown degradation peak at the retention time of 3.283 min in 6 % H_2O_2 at the end of 4 h. It can be understood that iloperidone is having good stability and degrading only at the extreme stressed conditions. (Figure 5)

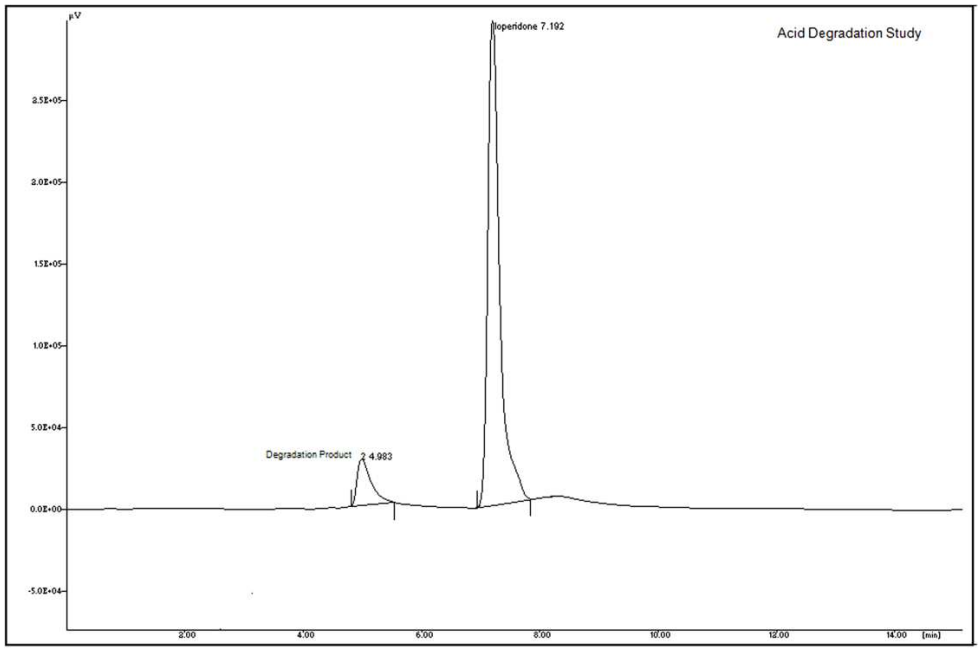

Figure 3 Typical chromatogram of acid stressed iloperidone

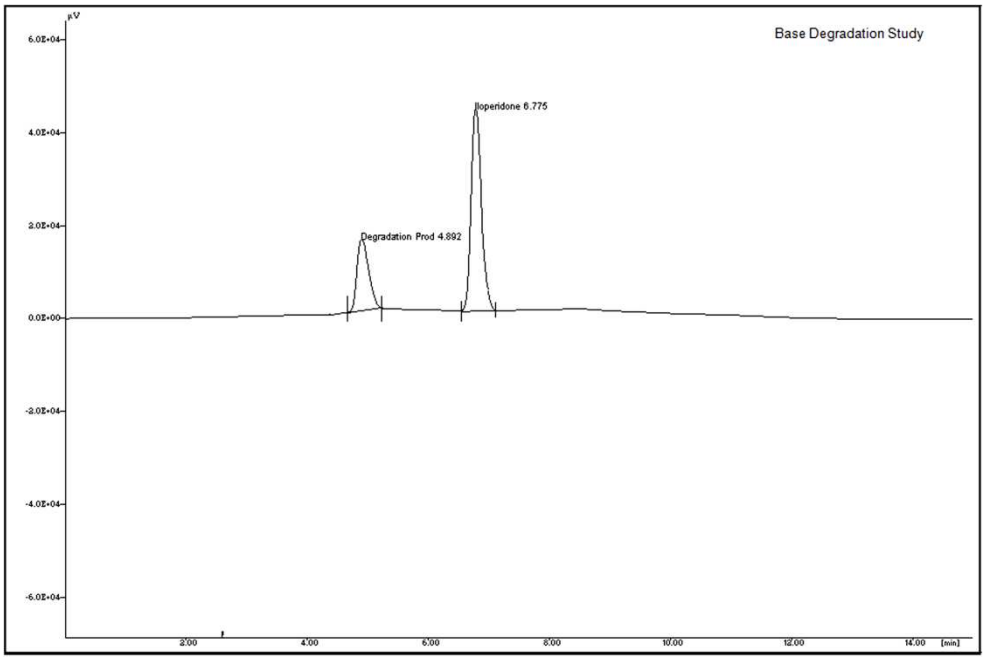

Figure 4 Typical chromatogram of base stressed iloperidone

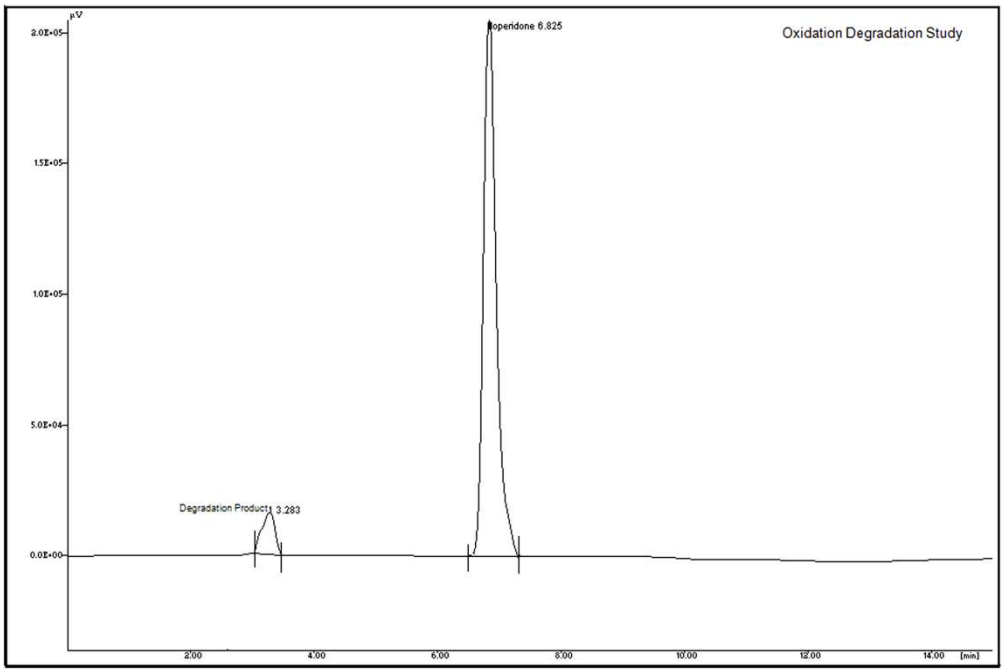

Figure 5 Typical chromatogram of H_2O_2 stressed chromatogram

Degradation was not observed in stressed conditions when the analyte was subjected to photolytic, thermal, and water hydrolysis i.e. neutral degradation studies.

CONCLUSION

HPLC method was developed and validated as per ICH guidelines. Accurate quantitation of chromophoric compounds was observed by UV detection. The drug was analysed by HPLC method using Thermo Hypersil ODS–C18 (250 mm × 4.6 mm, 5.0 µ) column from Germany with isocratic conditions and simple mobile phase containing acetonitrile:0.025M buffer: triethylamine (60:39.9:0.1) at flow rate of 1 mL/min using UV detection at 229 nm. The procedure has been evaluated for the linearity, accuracy, precision and robustness in order to ascertain the suitability of the analytical method. The method was also applied to marketed samples. It has been proved that the method is selective and linear between concentration range 100-600 ng/mL for iloperidone. LOD and LOQ were found to be 30 ng/mL and 60 ng/mL respectively.

Statistical analysis proved that the method is suitable for the analysis of iloperidone and as bulk drug and in pharmaceutical formulation without any interference from the excipients. The method has been successfully applied to stability study. Iloperidone was found unstable in acidic, alkaline and oxidative media but stable in neutral, dry heat and photolytic stressed conditions. This method may be further extended to study the estimation of drug and its metabolites in plasma and other biological fluids.

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