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Research Article

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A validated reverse phase high performance liquid chromatographic assay method for the estimation of iloperidone in bulk and commercial tablet dosage form

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ABSTRACT

A simple, rapid, accurate, precise and sensitive reverse phase HPLC method was developed and validated for the estimation of Iloperidone in bulk and pharmaceutical dosage form. The chromatographic separation was achieved by isocratic mode of elution by using Sunfire C_{18} (50 x 4.6 mm, particle size 3.5 µm) column and mobile phase consisting of phosphate buffer (0.02 M KH_2PO_4, adjusted to pH 3.65 with triethylamine) and acetonitrile in the ratio of 72:28 % v/v was used with a flow rate of 1 ml/min and the analyte was monitored at 230 nm. The retention time was found to be 3.185 min. The developed method was validated as per ICH guidelines. The calibration curve of Iloperidone was linear over the range of 7.5 - 45 µg/ml with correlation coefficient of 0.999. The limit of detection (LOD) and limit of quantification (LOQ) values were found to be 1.02µg/ml and 3.44 µg/ml respectively. The % recovery of Iloperidone in tablet dosage form was found to be 99.24 %. The method can be utilized for quantitative determination of Iloperidone in bulk and pharmaceutical dosage form.

Keywords: Iloperidone, RP-HPLC, Sunfire C_{18} Column, Validation, ICH guidelines.

INTRODUCTION

Iloperidone is chemically a (1-[4-[3-[4-(6-fluoro-2-benzisoxazol-3-yl)-1-piperidinyl]propoxy]-3-methoxyphenyl] ethanone) (Figure 1). It is a second generation atypical antipsychotic drug that belongs to the class of piperidinyl-benzisoxazole derivative used for the treatment of schizophrenia. Iloperidone is 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, namely, 1 mg, 2 mg, 4 mg, 6 mg, 8 mg, 10 mg and 12 mg. It has potent selective antagonist activity for the noradrenaline (α_{2C}), dopamine (D_{2A} and D_3) and serotonin (5-HT_{1A} and 5-HT_6) receptors [1].

In the literature review, a liquid chromatographic-mass spectrometric (LC-MS) method has been described by Mutlib et al., to quantitate Iloperidone and its principal metabolite 6-fluoro-3-(piperidin-4-yl)benzo-[*d*]-isoxazole hydrochloride and 1-(4-(3-chloropropoxy)-3-methoxyphenyl) ethanone in human plasma [2]. A derivative UV-spectrophotometric method was developed by Venkata Mahesh et al [3]. Determination of related substances of Iloperidone in bulk and dosage form by RP-HPLC was done by Naresh Chandra Reddy et al [4]. Manjula Devi et al. have reported validation of UV-spectrophotometric & HPLC method by using internal standard [5]. Stress degradation and development of stability indicating HPLC method was developed by Leenata P Mandpe et al [6]. The objective of this study was to develop a new, simple, rapid, precise, accurate, and specific RP-HPLC method as per ICH recommended conditions.

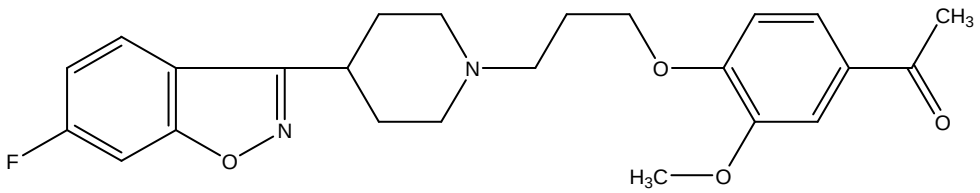

Fig. 1: Chemical structure of Iloperidone

EXPERIMENTAL SECTION

Chemicals and reagents

Iloperidone obtained as a gift sample from Sun Pharmaceuticals, Mumbai. Potassium dihydrogen orthophosphate, triethylamine (analytical grade), methanol and acetonitrile (HPLC grade) were supplied by SD fine chem. limited, Mumbai. Ortho phosphoric acid (analytical grade) and water (HPLC grade) were obtained from Rankem chemicals.

Instrumentation

Quantitative HPLC was performed on Waters alliance 2695 separation module containing PDA detector equipped with auto injector and Empower software. A reverse phase analytical column Sunfire C_{18} (50 x 4.6 mm, particle size 3.5 µm) was used.

Method development and optimization of chromatographic conditions

To develop a suitable RP-HPLC method for the determination of Iloperidone, different mobile phases like acetonitrile:water, phosphate buffer (pH 6.0):methanol, phosphate buffer (pH 3.65): methanol and phosphate buffer (pH 3.65):acetonitrile were tried at different flow rates of 0.8 and 1.0 ml/min. The mobile phase phosphate buffer (pH 3.65 adjusted with triethylamine): acetonitrile in the ratio of 72:28 % v/v at a flow rate 1.0 ml/min gave sharp peak with good symmetry. The retention time was found to be 3.185 min. The detection response was measured at 230 nm and column was maintained at ambient temperature throughout study. Optimized chromatographic conditions are given in Table 1.

Table-1: Optimized chromatographic conditions

Method parameters	Optimized condition
Column	Sunfire C_{18} (50 x 4.6 mm, particle size 3.5 µm)
Wavelength detection	230 nm
Mobile phase composition	0.02 M Potassium dihydrogen phosphate buffer (pH 3.65):acetonitrile (72:28 % v/v)
Pump mode	Isocratic
Flow rate	1.0 ml/min
Injection volume	10 µl
Run time	7 min

Preparation 0.02 M phosphate buffer:

Accurately weighed and transferred 2.72 gm of potassium dihydrogen orthophosphate in a 1000 ml of volumetric flask, 900 ml of Milli-Q water and 1 ml of triethylamine were added, filtered, sonicated for 5 min and finally made up the volume with water. Then pH was adjusted to 3.65 with dilute ortho phosphoric acid solution.

Preparation of standard stock solution:

Accurately 10 mg of Iloperidone was weighed and transferred into a 10 ml clean dry volumetric flask, 7 ml of diluent was added and sonicated for 30 min and made up to the final volume with diluent.

Preparation of working standard solution:

1 ml of above stock solution is diluted to 10 ml with diluent to obtain the concentration of 100 µg/ml of Iloperidone.

Diluent:

Methanol is used as diluent throughout the study.

Preparation of linearity solutions:

From the above working standard solution, calibration solutions were prepared by diluting aliquots of 0.075, 0.15, 0.225, 0.3, 0.375, 0.45 ml into 10 ml volumetric flasks. Then volume was made up with diluent to obtain the concentrations of 7.5, 15, 22.5, 30, 37.5 and 45 µg/ml of Iloperidone.

Preparation of sample solution for assay:
20 tablets were accurately weighed and crushed into fine powder. Then powder equivalent to 10 mg of Iloperidone was weighed and transferred into a 10 ml volumetric flask, 5 ml of diluent was added and sonicated for 5 min, further the volume was made up with diluent and filtered through 0.45 µ nylon membrane filter. From the above solution, 1 ml was pipette out and transferred into a 10 ml volumetric flask and then volume was made up with diluent.

Method validation
Validation of the proposed RP-HPLC method was carried out as per ICH guidelines by means of following parameters [7, 8].

Linearity and range
Aliquots of working standard solution was pipetted and diluted to final volume with diluent to obtain concentrations in the range of 7.5 - 45 µg/ml. Then 10 µl of each solution was injected in to the HPLC system under the optimized chromatographic conditions and the peak area responses were recorded. All the measurements were carried out three times for each concentration. Calibration curves for Iloperidone were plotted between peak area versus concentrations and regression equations were calculated.

Accuracy:
Accuracy was determined by adding the known amount of standard drug to the pre analyzed concentrations of assay samples by standard addition method. The recovery studies were carried out in triplicate of three different levels of 50 %, 100 % and 150 % by spiking standard drug solution to the sample.

Precision and system suitability:
The system and method precision of the proposed method is ascertained by injecting 6 replicates of standard and test sample in to the chromatographic system. The % recovery and % RSD were calculated. Intraday and interday precision provides an indication of its reliability.

LOD and LOQ
The sensitivity of HPLC was determined from LOD and LOQ which is calculated from the calibration curve using the following equations

$LOD = 3.3\ \boldsymbol{\sigma} / S$

$LOQ = 10\ \boldsymbol{\sigma} / S$

Where
σ = Standard deviation of y intercept of regression line
S = Slope of the calibration curve

Robustness
Robustness is the measure of the ability of an analytical method to remain unaffected by small but deliberate variations in method parameters, such as temperature change (± 2 °C), mobile phase composition (± 2 ml), flow rate (± 0.1 ml/min), and temperature (± 5 ^{o}C). The sample solution containing 30 µg/ml of Iloperidone was injected under the varied conditions and change in the peak area response was noted.

Specificity
According to ICH guidelines, specificity is defined as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products and matrix components. In the case of assay, demonstration of specificity requires that it can be shown that the procedure is unaffected by the presence of impurities or excipients.

RESULTS AND DISCUSSION

The proposed RP-HPLC method for the estimation of Iloperidone in tablet dosage form was carried out using 0.02 M potassium dihydrogen orthophosphate buffer adjusted to pH 3.65 with triethylamine and acetonitrile in the ratio of 72:28 % v/v as mobile phase and proposed method was validated as per ICH guidelines and results were reported below.

System suitability studies

The column efficiency, peak asymmetry and % RSD were calculated using standard drug solution of Iloperidone and the values obtained demonstrated the suitability of the system for analysis of Iloperidone in tablet dosage form. The results are reported in Table 2.

Table-2: System suitability parameters of Iloperidone

Parameter	Results
Retention time	3.185 min
Theoretical plates	2175
Tailing factor	1.15
Peak area	1657135
% RSD	0.18

Linearity

The calibration curve was found to be linear over the concentration range of 7.5 - 45 µg/ml (as shown in Figure 2). The correlation coefficient was found to be 0.999.

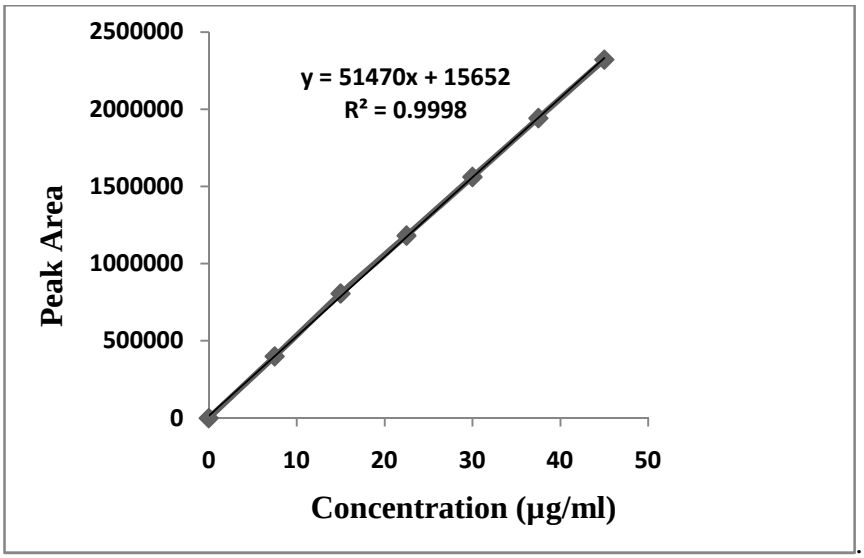

Fig. 2: Calibration curve of Iloperidone

Precision

The proposed method was found to be highly precise as the % RSD values of repeatability, intraday and interday studies were found to be below 2 % which is under the limit as per recommendations of ICH guidelines. The low % RSD values indicate the proposed method was very precise. The results are reported in Table 3.

LOD and LOQ

LOD and LOQ values of Iloperidone were determined from the calibration curve. LOD and LOQ were found to be 1.02 µg/ml and 3.44 µg/ml respectively. The results are shown in Table 3.

Analysis of Marketed Formulation

Tablet dosage form was analyzed and the results of assay showed that the amount of Iloperidone was in good agreement with the label claim of formulation as indicated by % assay which was found to be 99.24 % for Iloperidone. The retention times were found to be 3.185 min and 3.190 min for standard and sample solution of Iloperidone as shown in Figure 3 and Figure 4. All the results were found to be within the limits and therefore the proposed method was found to be free from interferences from excipients.

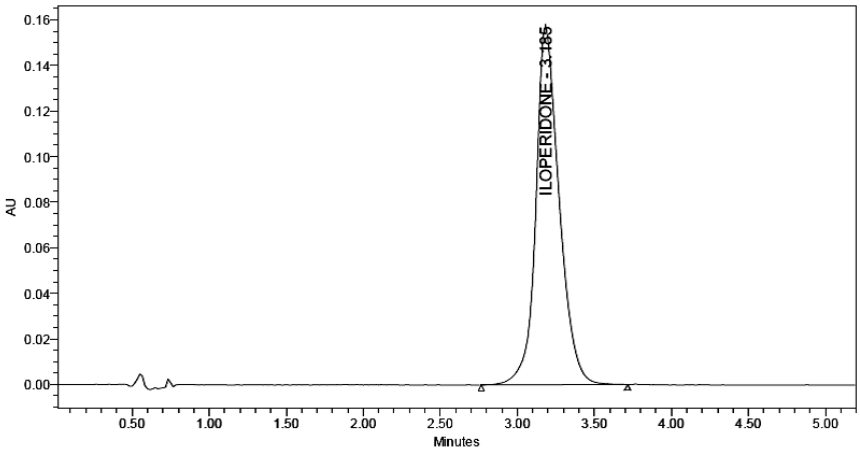

Fig. 3: A typical chromatogram of standard containing Iloperidone

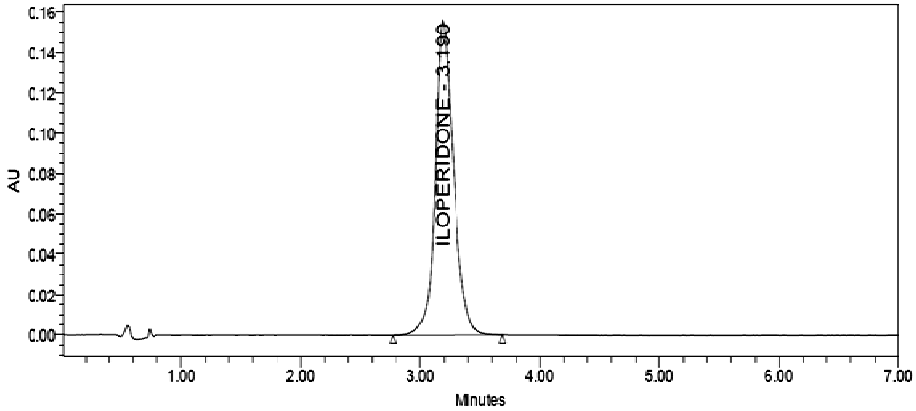

Fig. 4: A typical chromatogram of sample containing Iloperidone

Table-3: Validation parameters of the proposed RP-HPLC method

Parameter	Iloperidone
Linearity range	7.5 - 45 µg/ml
Regression equation	Y = 51470x + 15652
Correlation coefficient (r)	0.999
LOD (µg/ml)	1.00 µg/ml
LOQ (µg/ml)	3.04 µg/ml
Repeatability (% RSD, n=6)	0.18
Intraday precision (% RSD, n=3)	0.2 - 0.7
Interday precision (% RSD, n=3)	0.2 - 0.4

Accuracy

The % recovery of Iloperidone was found to be 99.68 - 100.73 %. Hence the proposed RP-HPLC method was said to be accurate. The results are shown in Table 4.

Table-4: Recovery studies

Drug	Level	Amount of sample drug taken (µg/ml)	Amount of standard drug spiked (µg/ml)	Mean % recovery* ± SD	% RSD
Iloperidone	50 %	30	15	99.68 ± 0.5851	0.59 %
	100 %	30	30	100.11 ± 0.7555	0.75 %
	150 %	30	45	100.73 ± 0.2713	0.27 %
** Mean of three determinations*					

Robustness

The developed method is robust with deliberate changes with variation of mobile phase composition, flow rate and temperature as % RSD shows below 2 with meeting system suitability parameters as per ICH guidelines. The results are given in Table 5.

Table-5: Results of robustness study

S. No	Parameter	Level	Retention time (min)	USP plate count	USP tailing	% RSD
1.	Flow rate	0.8	3.93	4343	1.08	1.3
	(± 0.2 ml/min)	1.2	2.72	4242	1.06	0.1
2.	Mobile phase composition	70: 30	2.87	4255	1.10	1.2
	(± 2 % v/v)	74: 26	4.08	4275	1.08	0.7
3.	Temperature	25 ℃	3.21	4336	1.06	1.2
	(± 5 ℃)	35 ℃	3.05	4294	1.05	0.4

CONCLUSION

The developed and validated RP-HPLC method was found to be rapid, accurate, precise and reliable which is useful for routine quantification in quality control analysis of Iloperidone in pharmaceutical dosage form. The system suitability parameters indicate good sensitivity, more ruggedness and robustness of the method. Therefore the proposed method has proven to be simple, selective and specific meeting the ICH guidelines for analytical method validation.

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REFERENCES

[1] JP Kelleher; F Centorrino; MK Albert; RJ Baldessarini. *CNS Drugs*, **2000**, 16(4), 249-261.
[2] AE Mutlib; JT Strupczewski. *J. Chromatography Biomed. Appl*., **1995**, 669(2), 237-246.
[3] R Venkata Mahesh; R Venkatesha Perumal; C Jose Gnana Babu; R Revathi; S Muneer; KP Channabasavaraj. *American Journal Pharm. Tech. Research*, **2011**, 1(4), 311-316.
[4] C Naresh Chandra Reddy; KB Chandra Sekhar. *International Journal of Pharmacy and Biological Sciences*, **2012**, 2(2), 208-217.
[5] AS Manjula Devi; TK Ravi. *International Journal of Pharma. Tech. Research*, **2012**, 4(2), 576-581.
[6] P Leenata Mandpe; B Varsha Pokharkar. *Pelagia Research Library*, **2011**, 2(2), 230-239.
[7] International conference on harmonization (ICH), Draft guidelines on validation of analytical procedure, Definition and terminology, Federal register. **1995**, 60, 11260.
[8] Validation of analytical procedure: Methodology Q2B, ICH harmonized tripartite guidelines, **1996**, 6-12.