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APPLICATION OF QUALITY BY DESIGN APPROACH FOR DEVELOPMENT OF ANALYTICAL PR-HPLC METHOD FOR ESTIMATION OF ILOPERIDONE IN API AND ITS BULK DOSAGE FORM

Rutuja S. Aher[1]*, Shekhar B. Kale[1]* and Deeliprao V. Derle[1]

Department of Quality Assurance, MVP Samaj's College of Pharmacy, Nasik, Maharashtra, India.

***Corresponding Author: Rutuja S. Aher**
Department of Quality Assurance, MVP Samaj's College of Pharmacy, Nasik, Maharashtra, India.

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ABSTRACT
The aim of the present study was to facilitate investigating the determination of Iloperidone in bulk drug and its pharmaceutical formulations by a developed and validated rapid reverse phase high performance liquid chromatography (RP-HPLC) method. The method was developed using QbD principles and design space (method operable design region) is defined. Suitable working point within design space was selected for validation and validated as per current guidelines. The calibration was linear in the range of 20-120 µg/ml. The relative standard deviation (RSD) percentage for accuracy, precision and robustness was observed within the range of 0.1 and 2.0. Hence, the method is found to be accurate, precise and robust. Accuracy carried by recovery method. System suitability parameters like asymmetry factor and theoretical plate number were found to be within USP acceptance criteria. The validated limit of quantitation (LOQ) 9.6114 µg/ml and limit of detection (LOD) of 3.17 µg/ml are low enough to allow determination of low concentrations of the drug. The assay result of marketed formulation was found to be 99.351%. The proposed method can be used for routine analysis in quality control laboratories for its bulk and formulated product.

KEYWORDS: HPLC, Iloperidone, Quality by Design, Validation.

INTRODUCTION[11]
Iloperidone is white crystalline powder having chemical name 1-[4-[3-[4-(6-fluoro-1,2-benzisoxazol-3-yl)-1-piperidinyl]propoxy]-3-methoxyphenyl]ethanone (Fig.-1), an atypical antipsychotic for the treatment of schizophrenia approved by the U.S. Food and Drug Administration (FDA)1-3 for use in the United States on May 6, 2009. It is used to treat the symptoms of schizophrenia (a mental illness that causes disturbed or unusual thinking, loss of interest in life, and strong or inappropriate emotions).

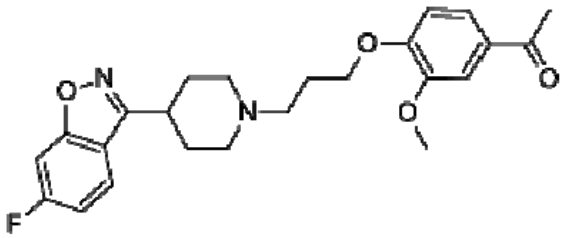

Fig. 1: Chemical structure of Iloperidone.

Iloperidone belongs to the subclass of combined D2/5HT2A antagonism, with greater affinity forthe 5HT2A receptor than D2 receptor antagonism. It use for acute treatment of schizophrenia in adults.

A few methods were reported in the literature for the estimation of Iloperidone that include Determination of related substances of Iloperidone in bulk and dosage form by RP-HPLC was done by Naresh Chandra Reddy et al[18], Lakshmi.B.et al[14], Usmangani K.Chhalotiya[13], G.Sarvanan[19] and Karaca et al 2017.[20] Manjula Devi et al. have reported validation of UV-spectrophotometric & HPLC method by using internal standard.[17] Stress degradation and development of stability indicating HPLC method was developed by Leenata P Mandpe et al.[16] Development and validation of RP-UPLC method

for the determination of iloperidone, its related compounds and degradation products in bulk and dosage form by Shashikant B.Landge.[15]

The objective of study was to develop a new, simple, sensitive, precise and accurate RP-HPLC method for Iloperidone in bulk and tablet dosage forms.

MATERIALS AND METHODS

API: Iloperidone was kindly procured as gift sample from Megafine pharmaceuticals, Nashik. Potassium dihydrogen orthophosphate (analytical grade), methanol (HPLC grade), Ortho phosphoric acid (analytical grade) and water (HPLC grade) was purchased from Modern chemical laboratory, Nashik, Maharashtra, India.

Instruments: For analytical purpose HPLC was performed on Waters 1525 separation module containing Waters 2489 (UV-Visible Detector) equipped with mannual injector and Breeze 2 software. A reverse phase analytical column Xtera C18 (250 x 4.6 mm, particle size 5 µm) was used.

Experimental Work

Method Development by QbD approach and optimization of chromatographic conditions

To develop a suitable RP-HPLC method for the determination of Iloperidone, different mobile phases like methanol :water(80-20), methanol :water(80-20) (pH 3.0), methanol, phosphate buffer 20mM(pH 3.0) were tried at different flow rates of 0.8 and 1.0 ml/min. The mobile phase phosphate buffer(pH 3.0 adjusted with): Ortho phosphoric acid methanol in the ratio of 80:20% v/v at a flow rate 0.8ml/min gave sharp peak with good symmetry. The retention time was found to be 3.82 min. The detection response was measured at 227 nm and column was maintained at ambient temperature throughout study. Optimized chromatographic conditions are given in Table 2.

Design of experiment[1,2,3,4,5,6,7]

3^3 randomized response surface designs with a Box-Behnken design were used with 17 trial runs to study the impact of three factors on the two key response variables. In this design 3 factors were evaluated, each at 3 levels, and experimental trials were performed at all 3 possible combinations. The mobile phase compositions (X1), pH(X2) & flow rate (X3) were selected as independent variables and retention time (RT), theoretical plate number (TPN) & asymmetry factor were selected as dependent variables. The resulting data were fitted into Design Expert 11 software and analysed statistically using analysis of variance (ANOVA). The data were also subjected to 3-D response surface methodology to determine the influence of flow rate, pH, and mobile phase composition on dependent variables.

Table no. 1: Levels selected.

Level of Variable	Levels of Factors		
	Mobile Phase Composition (%v/v)	pH	Flow rate (ml/min)
Low Level (-1)	70	3.0	0.8
Medium Level (0)	80	3.5	0.9
High Level (1)	90	4.0	1.0

Table 2: Optimized chromatographic conditions.

Method parameters Optimized condition
Column Xtera C18 (250 x 4.6 mm, particle size 5 µm)
Wavelength detection 227 nm
Mobile phase composition 10mM Potassium dihydrogen phosphate buffer (pH3.0):methanol(80:20%v/v)
Pump mode Isocratic
Flow rate 0.8 ml/min
Injection volume 10 µl
Run time 7 min

Preparation 0.01M phosphate buffer

Accurately weighed and transferred 1.36 gm of potassium dihydrogen orthophosphate in a 1000 ml of volumetric flask, 900 ml of HPLC water and were added, filtered, sonicated for 5 min and finally made up the volume with water. Then pH was adjusted to 3.0 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 and made up to the final volume with diluent.

Preparation of linearity solutions

From the above standard solution, calibration solutions were prepared by diluting aliquots of 0.2, 0.4,0.6, 0.8, 1.0, 1.2 ml into 10 ml volumetric flasks. Then volume was made up with diluent to obtain the concentrations of 20,40,60,80,100 and 120 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[10]

The proposed method was validated according to ICH Q2 (R1) guidelines for validation of analytical procedures. As per the ICH guidelines the method was validated for Linearity, Precision, Accuracy, Limit of Detection, Limit of Quantification Robustness and Ruggedness.

Linearity and range

Aliquots of working standard solution was pipetted and diluted to final volume with diluent to obtain concentrations in the range of 20 - 120 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.

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.

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 80%, 100% and 120% by spiking standard drug solution to the sample.

Limit of Detection and Limit of Quantification

The Limit of Detection (LOD) is the smallest concentration of the analytes that gives the measurable response. LOD was calculated using the following formula

$LOD = 3.3\ \sigma / S$

The Limit of Quantification (LOQ) is the smallest concentration of the analytes, which gives response that can be accurately quantified. LOQ was calculated using the following formula

$LOQ = 10\ \sigma / S$

Where, σ is standard deviation of the response and S is the slope of the calibration curve.

Robustness

In robustness the capacity of method to remain unaffected by the small deliberate changes is calculated. The study includes change of wavelength of maximum absorbance and analysing the peak area response. The results are interpreted with respect to relative standard deviation.

RESULT AND DISCUSSION

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 3.

Table 3: System suitability parameters of Iloperidone.

Parameter Results
Retention time 3.815 min
Theoretical plates 2532
Tailing factor 1.03
Peak area 2210919.2
% RSD 1.18

Linearity

The calibration curve was found to be linear over the concentration range of 20 - 120 µg/ml (as shown in Figure 2). The correlation coefficient was found to be 0.999.

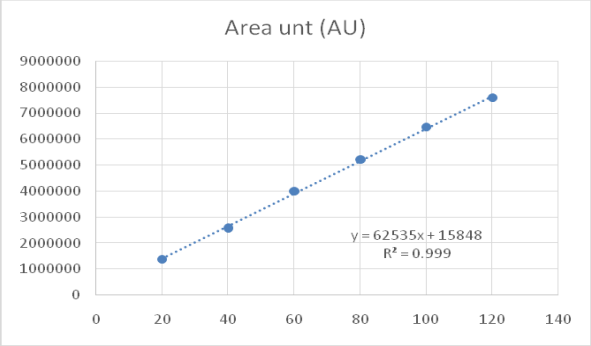

Fig. 2: Calibration curve of Iloperidone.

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 4.

LOD and LOQ

LOD and LOQ values of Iloperidone were determined from the calibration curve. LOD and LOQ were found to be 3.17 µg/ml and 9.6114 µg/ml respectively. The results are shown in Table 4.

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 98.65% for Iloperidone. All the results were found to be within the limits and therefore the proposed method was found to be free from interferences from excipients.

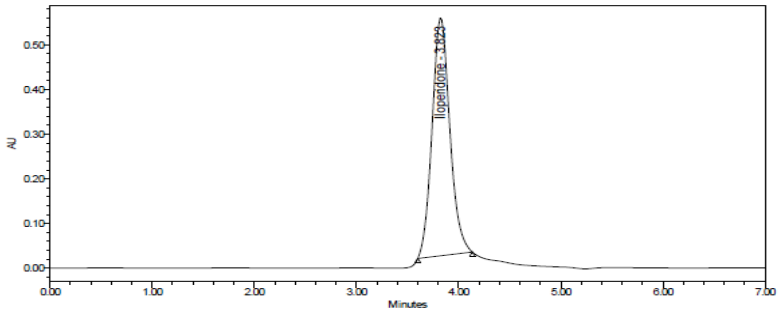

Fig. 3: A typical chromatogram of standard containing Iloperidone.

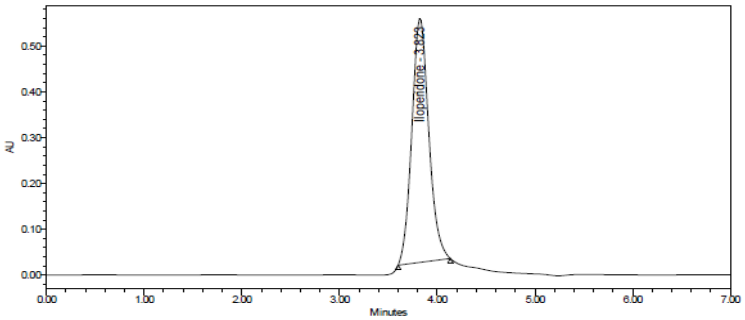

Fig. 4: A tyoical chromatogram of sample containing Iloperidone.

Table 4: Validation parameters of the proposed RP-HPLC method.

Parameter Iloperidone
Linearity range 20 - 120 µg/ml
Regression e equation Y = 62535x + 15848
Correlation c coefficient (r) 0.999
LOD (µg/ml) 3.17 µg/ml
LOQ (µg/ml) 9.6114 µg/ml
Repeatability(% RSD, n=6) 0.942
Intraday precision (%RSD, n=3) 1.18
Interday precision (% RSD, n=3) 1.04

Accuracy

The accuracy studies were performed by using 80%, 100% and 120% of the solution 0f 100ppm. The % recovery of Iloperidone was found to be 99.68 -to be accurate. Hence the propose RP-HPLC method was said to be accurate. The results are shown in Table 5.

Table 5: Recovery studies.

Drug	Level	Amount of sample drug taken (µg/ml)	Amount of standard drug spiked (µg/ml)	Mean % Recovery	%RSD
Iloperidone	80%	50	40	98.315	0.6519
	100%	50	50	99.13	0.7686
	120%	50	60	99.30	0.4011

*** Mean of three determinations**

Robustness

The developed method is robust with deliberate changes with variation of wavelength of maximum absorbance and analysing the peak area response as % RSD shows below 2 with meeting system suitability parameters as per ICH guidelines. The results are given in Table 6.

Table 6: Results of robustness study.

ILOPERIDONE				
Change in wavelength(±)	**Retention Time (min)**	**Area**	**Plate Count**	**Tailing factor**
225	3.808	6420104	2453.1	1.145
225	3.808	6420630	2457.19	1.077
227	3.839	6443183	2312.50	1.207
227	3.839	6443182	2189.50	1.20
229	3.804	6421956	2312	1.124
229	3.804	6421956	2421	1.118
MEAN		6224963.66	2357.54	1.145
SD		16141.320		
%RSD		0.259		

CONCLUSION

The RP-HPLC method was developed and it is found to be simple, accurate, precise, highly sensitive, reproducible and inexpensive. The proposed method was found suitable for determination of Iloperidone in API and its bulk dosage form without any interference from the excipients. The validation procedure confirms that this is a workable method for their quantification in the raw material and also in the formulations. Hence it can be effectively applied for the routine analysis of Iloperidone in bulk drug. Its advantages are low cost of reagents, speed and simplicity of sample treatment, satisfactory precision and accuracy.

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