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NEW RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF ILOPERIDONE IN BULK DRUG AND ITS PHARMACEUTICAL DOSAGE FORM

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ABSTRACT
The aim of the present study was to develop and validate a simple RP-HPLC method to determine Iloperidone in bulk and Pharmaceutical Dosage Forms. The method employed, with PEAKHPLC instrument with LiChrospher C18 (250 mm x 4.6 mm, 5µ) column with reverse phase isocratic pump mode, Mobile phase consists of Acetonitrile , Methanol, acetate buffer in the ratio of 50:30:20(v/v/v) with flow rate 0.9 ml/min. Detection was carried out at 215nm.The linear range and correlation coefficient (r2) was found 20-100µg/mland 0.999 respectively. The value of correlation coefficient was found to be 0.999. The recovery of Iloperidone was about 98.82-100.48%.The LOD and LOQ values for were found to be 0.01 and 0.03 µg/ml respectively.The proposed method was found to be simple,precise, suitable and accurate for quantification of Iloperidone in bulk drug and tablet dosage form.
Keywords: Iloperidone, RP-HPLC, Method validation.

INTRODUCTION

Chemically Iloperidone is 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).

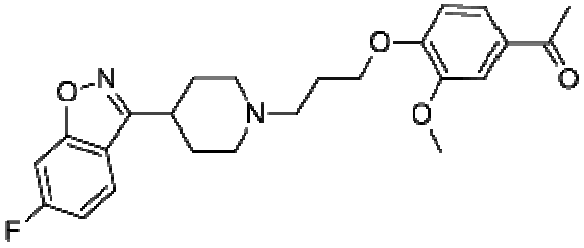

Fig.-1: Chemical structure of Iloperidone

Iloperidone is a monoamine directed towards acting upon and antagonizing specific neurotransmitters, particularly multiple dopamine and serotonin receptor subtypes. In addition, pharmacogenomic studies identified single nucleotide polymorphisms associated with an enhanced response to iloperidone during acute treatment of schizophrenia[4-7] .

A few methods were reported in the literature for the estimation of Iloperidone(8-18),UV spectrophotometric[8-9],HPLC[9-13],LC-MS[14-17],HPLC-MS/MS[18] .In the present study the authors reported a rapid, precise, specific, robust, economical and accurate HPLC method for the quantification of Iloperidone in bulk and tablet dosage forms.

EXPERIMENTAL

Chromatographic conditions

The analysis of the drug was carried out on PEAKHPLC instrument with LiChrospher C18 column, LC 20AT pump for solvent delivery and variable wavelength programmable LC – 7000 UV-detector. A 20µL Rheodyne inject port was used for injecting the samples. Data was analyzed by using PEAK software.

Determination of wavelength of maximum absorbance

The standard solutions were scanned in the range of 200 -400nm against mobile phase as a blank. Iloperidone showed maximum absorbance at 215nm. So the wavelength selected for the determination of Iloperidone was 215nm.

Chemicals and solvents

Working standard of Iloperidone gifted from well reputed research laboratories. Acetonitrile, Methanol, acetate buffer used were of HPLC grade and purchased from Merck Specialties Private Limited, Mumbai, India. All other chemicals were also purchased from Merck Specialties Pvt. Limited, Mumbai.

Preparation of standard stock solution

Standard stock solution of Iloperidone pure drug (1mg/ml) was prepared by accurately weighing about 10mg of drug in 10ml volumetric flask. The drug was dissolved with few ml of acetonitrile, and sonicated to dissolve it completely and made up to the mark with the same solvent. The contents were mixed well and filtered through 0.45µm nylon membrane filter paper. From this stock solution 60ppm standard solution was prepared by diluting standard stock solution with the same solvent.

Preparation of sample solution

Iloperidone tablets are purchased from local market. 20 tablets of Iloperidone were powered and the average weight of the powder was calculated. From the powder, an equivalent weight of 10mg of Iloperidone was weighed and was dissolved in little amount of acetonitrile and mixed well and then make up to 10ml with acetonitrile. It was filtered through 0.45µm nylon membrane filter paper. From this stock solution 60ppm standard solution was prepared by diluting standard solution with the same solvent. This solution was used for formulation estimation.

HPLC method development and optimization:

To optimize the chromatographic conditions, different combinations of Acetonitrile, Methanol, and Acetate Buffer were tested. The separation, well resolved and good symmetrical peaks were obtained with the mobile phase Acetonitrile: Methanol: Acetate Buffer in the ratio of 50:30:20v/v/v. The retention time of Iloperidone was found to be 6.57 min with pump pressure 5.9±5MPa, which indicates a good retention time. The method found to be specific. The percentage of recoveries were in the range of 98.82-100.48%, indicating that the proposed method is highly accurate and the proposed liquid chromatographic method was applied for the determination of Iloperidone in bulk and tablet formulations. System suitability parameters and Chromatographic Conditions of Iloperidone were given in Table 1. Standard, blank and formulation chromatograms were shown in figures-2, 3 and 4.

Table-1: Table showing optimized chromatographic conditions

S.No.	Parameter	Condition
1.	Mobile phase	Acetonitrile: Methanol: Acetate Buffer 50: 30:20 (v/v)
2.	Pump mode	Isocratic
3.	pH	4.1 with Acetic Acid
4.	Column	LiChrospher C18 column (250 X 4.6 mm, 5µ)
5.	Column Temp	Ambient
6.	Wavelength	215nm
7.	Injection Volume	20µl

8.	Flow rate	0.9m/min
9.	Run time	12minutes
10.	Retention Time	6.57min
11.	Theoretical plates	7186
12.	Tailing Factor	1.44
13.	Pump Pressure	5.9±5MPa

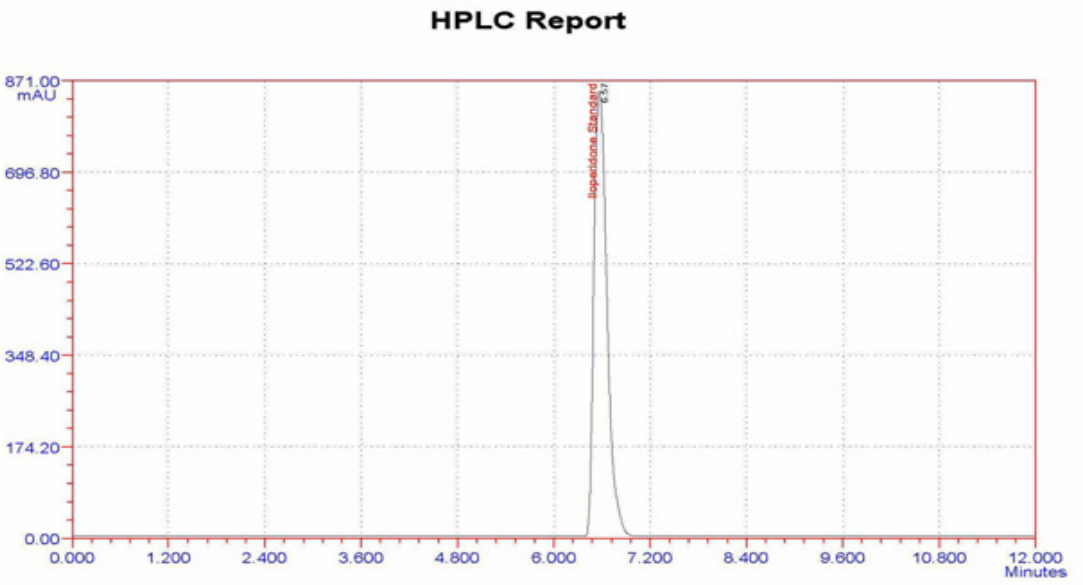

Fig.-2: Standard chromatogram of Iloperidone

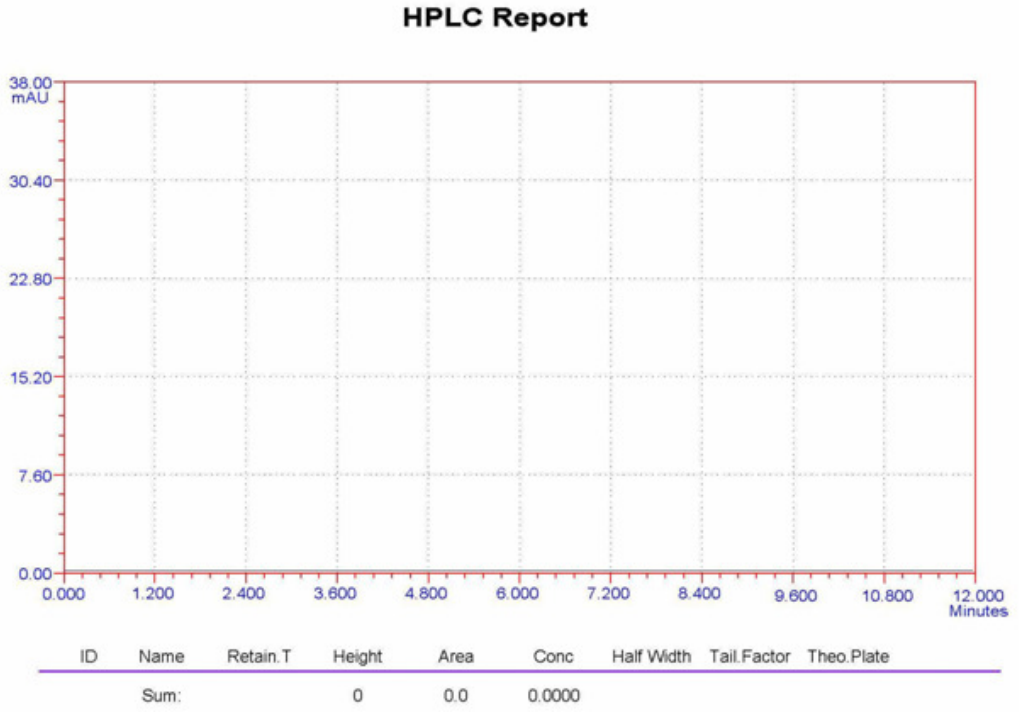

Fig.-3: Blank chromatogram for Iloperidone

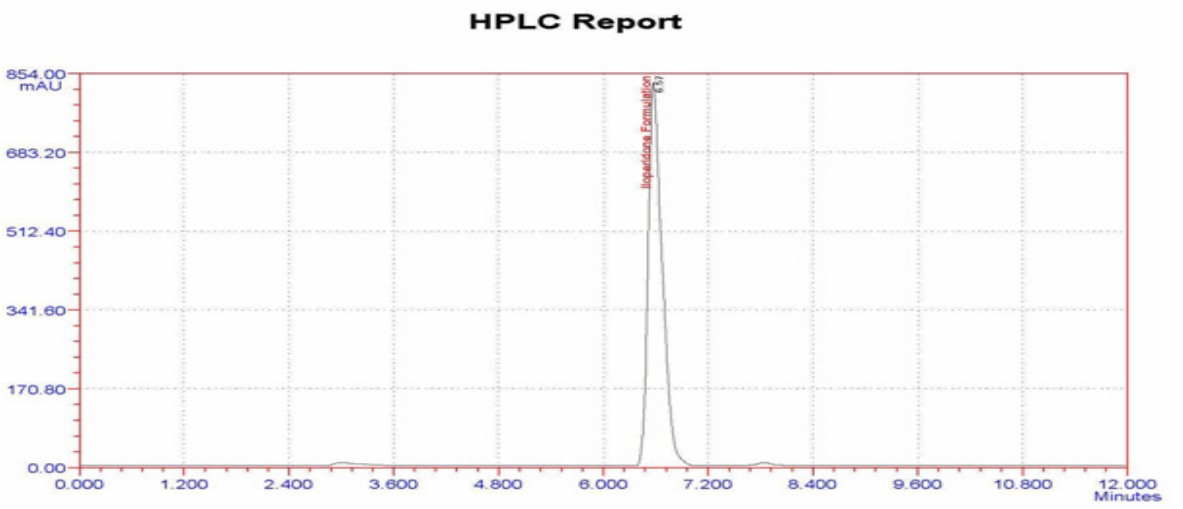

Fig.-4: Sample chromatogram

Method Validation

The objective of the method validation is to demonstrate that the method is suitable for its intended purpose as it is stated in ICH (International Counsel of Harmonization) guidelines for various parameters like linearity, precision, system suitability, specificity, limit of detection and limit of quantification and robustness.

Linearity and Range

Linearity was done by using prepared standard solutions of nine different concentrations levels ranging from 20 to 100 µg/ml that were injected into the HPLC column, keeping the injection volume constant and Chromatograms were recorded at 215 nm. Calibration curve was plotted between the mean peak area vs. respective concentration (Table 2&Figure 5). The regression equation of calibration curves was obtained as y = 11945x + 195693 with a correlation coefficient of 0.999. Where slope (m) is 11945 and intercepts (c) is 195693. Results of linearity studies are given in Table 2 and calibration curve was shown in figure 3.

Table-2: Linearity results for Iloperidone

S.No.	Concentration in µg/ml	Peak Area
1	20	441313
2	30	530370
3	40	675710
4	50	809449
5	60	914811
6	70	1039730
7	80	1141046
8	90	1273870
9	100	1385184
Slope: 11945 Intercept: 19569 Correlation Coefficient:0.9989		

Limit of Detection (LOD) and Limit of Quantitation (LOQ): The LOD and LOQ were calculated by mathematical equation. LOD= 3.3×standard deviation ÷ slope and

LOQ=10×standard deviation ÷ slope. The LOD was found to be 0.01µg/ml and the LOQ was found to be 0.03 µg/ml.

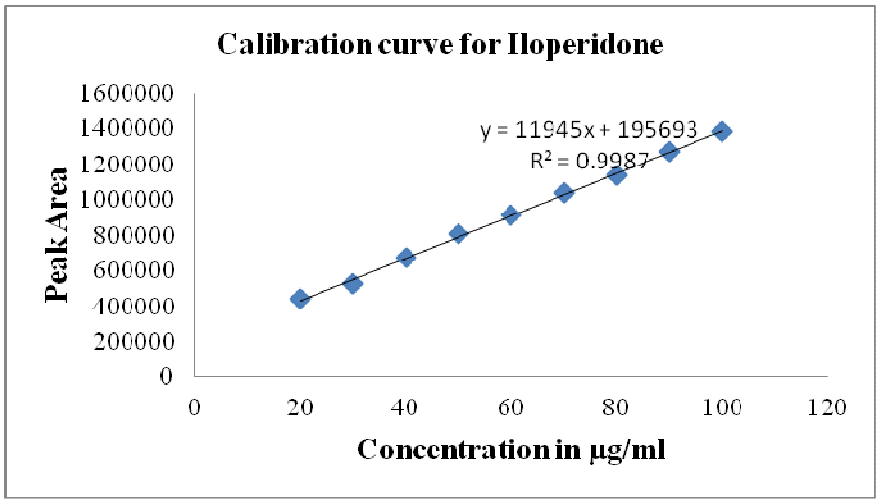

Fig.- 5: Calibration curve for Iloperidone

Precision: Six standard solutions of **Iloperidone** drug (60 ppm) were prepared and analyzed. The percentage of relative standard deviation (% RSD) for responses of peak was calculated and it was found less than 2%. Results of precision studies are shown in Table 3(a), 3(b).

Table-3(a): Intraday precision results

S.No.	Concentration in µg/ml	Peak Area
1	60	919496
2	60	924912
3	60	911899
4	60	911704
5	60	915732
6	60	919017
RSD: 0.55		

Table-3(b): Interday precision results

S.No.	Concentration in µg/ml	Peak Area
1	60	910670
2	60	907477
3	60	914008
4	60	917759
5	60	913257
6	60	911544
RSD: 0.38		

LOD and LOQ

The Limit of Detection (LOQ) is the smallest concentration that can be detected but not necessarily quantified as an exact value. The LOD value is 0.01µg/ml.The Limit of Quantification of (LOQ) is the lowest amount of analyte in the sample that can be quantitatively determined with suitable precision and accuracy. LOQ observed is 0.01µg/ml and the values are given in Table-4

Table-4: LOD and LOQ results for the proposed method

S. No.	Test	Results
1	LOD	0.01µg/ml
2	LOQ	0.03µg/ml

Robustness

The robustness of the method was evaluated by varying the three of the chromotographic conditions those are mobile phase composition, pH and wavelength of the detector. The %of change in the proposed method ws found to be with in the range of 0.42-1.84. The results showed that the method is robust and the small deliberate changers didn't effect either the peak shape or other optimized chromotographic parameters. robustness results were given in Table-5.

Table-5: Table showing the results of robustness

S.No.	Parameter	Change	Area	% of Change
1	Standard	-	914811	-
2	MP 1	55: 25:20 (v/v)	907203	0.83
2	MP 2	45: 35:20 (v/v)	931686	1.84
3	pH 1	4.0	924144	1.02
4	pH 2	4.2	919365	0.50
5	WL 1	212nm	919051	0.46
6	WL 2	218nm	910982	0.42

Recovery studies

Recovery test was performed at three different concentrations 30µg/ml, 60 µg/ml, 90 µg/ml. Results were given in Table-6.

Table-6: Showing the results of Recovery

% of Recovery	Spiked conc., (µg/ml)	% of Assay
50%	30	99.19
	30	98.94
	30	99.62
100%	60	99.12
	60	98.82
	60	98.95
150%	90	99.15
	90	99.66
	90	100.48

Formulation analysis

The formulation solution was injected 99.47% assay was observed for the proposed method. Results were shown in Table-7 and formulation chromatogram was shown in figure-6.

Table-7: Formulation results for Iloperidone

S.No.	Brand name	Available form	Label claim	Concentration	Amount found	% Assay
1	Fanapt	Tablet`	2mg	60µg/ml	59.68µg/ml	99.47

Analysis of drug sample

A sample solution of 60µg/ml was prepared using bulk drug of Iloperidone. The prepared solution was injected in the operating conditions of the optimized conditions; peak area response was used for calculating the assay. The % assay in the bulk drug analysis was found to be 98.01%. This confirms that the method was applied for the estimation of Iloperidone in bulk drug also. The chromatogram of bulk drug was shown in figure-6.

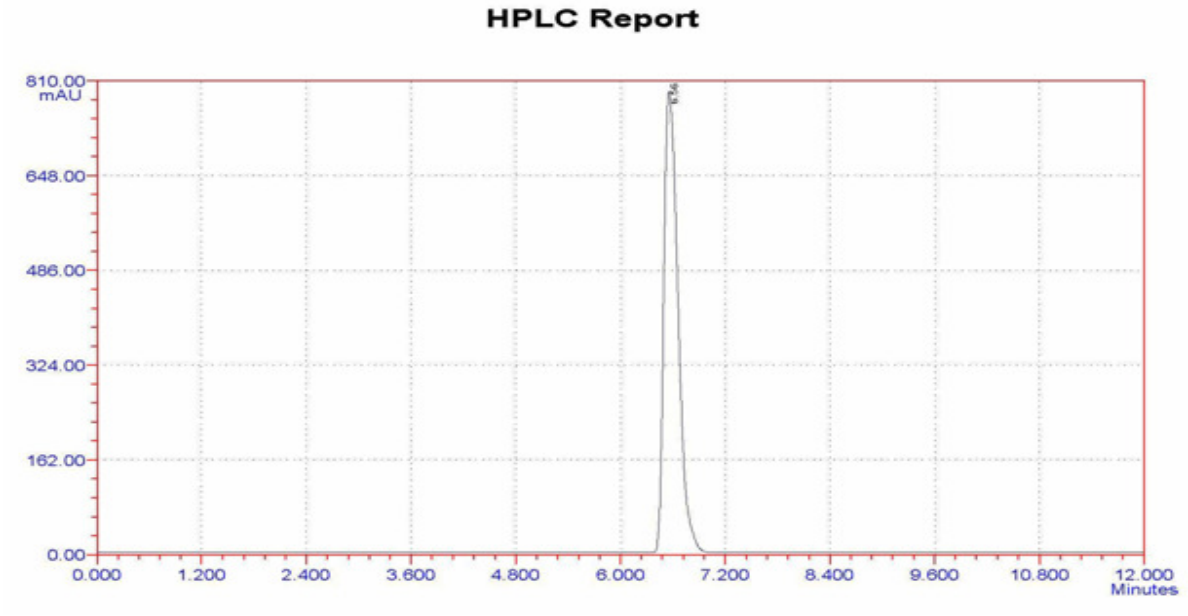

Fig.-6: Drug chromatogram of Iloperidone

To study the application of proposed method it was applied for assay of commercial tablets of Iloperidone and bulk drug sample. The results 98.76% for formulation presented good agreement with the labeled content and was found to be 98.05% results were observed. This confirms that the proposed method can be applicable for the estimation of Iloperidone in drug product also. Thus, the system meets suitable criteria.

CONCLUSION

In the present work, an attempt has been made to develop the method using RP HPLC method for estimation of Iloperidone in combined dosage form with simple, accurate, specified with less retention times. The mobile phase is simple to prepare and economical. The proposed method was found to be rapid, accurate, precise, specific, robust and economical and complies system suitability limits. The RSD indicates the method is precise and accurate, the mean recoveries were found in the range of 98-102%. The sample recoveries in all formulations were in good agreement with their respective label claims and they suggested non-interference of formulation excipients in the estimation. Thus the method is not time consuming and can be used in laboratories for the routine analysis. The method was also specific, stable and robust. The proposed method was validated in accordance with ICH parameters and the results of all methods were very close to each other as well as to the label value of commercial pharmaceutical dosage form and bulk drug.

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