A NOVEL RP-HPLC METHOD FOR THE QUANTIFICATION OF ILOPERIDONE IN FORMULATIONS

BRC Sekhar Reddy, Dr. Nallagatla. Vijaya Bhaskar Rao*

Micro Labs Limited-API, # 43-45 KIADB Industrial Area Phase-IV, Bommasandra, Bangalore- 560 105.

Email : brcsekharreddy@gmail.com

ABSTRACT

A simple, precise and accurate RP-HPLC method was developed and validated for rapid assay of Iloperidone tablet dosage form. Isocratic elution at a flow rate of 1ml/min was employed on a Zodiac C18 (250x4.6mm, 5µm in particle size) at ambient temperature. The mobile phase consisted Methanol: Acetonitrile 80:20 v/v. The UV detection wavelength was 210nm and 20µl sample was injected. The retention time for Iloperidone was 7.1min. The percentage RSD for precision and accuracy of the method was found to be less than 2%. The method was validated as per the ICH guidelines. The method was successfully applied for routine analysis of Iloperidone tablet dosage form and bulk drug.

KEY WORDS Iloperidone, RP-HPLC, UV detection, recovery, precise, 210nm

INTRODUCTION

Iloperidone, is an atypical antipsychotic for the treatment of schizophrenia (a mental illness that causes disturbed or unusual thinking, loss of interest in life, and strong or inappropriate emotions) previously known as Zomaril,. It was approved by the U.S. Food and Drug Administration (FDA) for use in the United States on May 6, 2009. [1] It is belonging to the chemical class of piperidinyl-benzisoxazole and it works by changing the activity of certain natural substances in the brain.

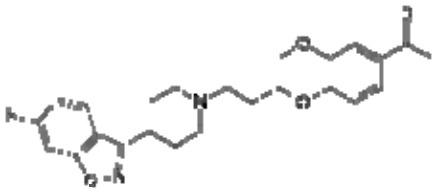


Fig: 1 Structure of Iloperidone

Systematic (IUPAC) name	:	1-[4-[3-[4-(6-fluoro-1,2-benzisoxazol-3-yl)-1-piperidinyl]propoxy]-3-methoxyphenyl]ethanone
Formula	:	$C_{24}H_{27}FN_2O_4$
Routes	:	Oral, injection
Mol. mass	:	426.481g/mol

Iloperidone has been shown to act as an antagonist at all tested receptors. It was found to block the sites of noradrenaline (α_{2C}), dopamine (D_{2A} and D_3), and serotonin (5-HT_{1A} and 5-HT_6) receptors.[2] In addition, pharmacogenomic studies identified single nucleotide polymorphisms associated with an enhanced response to iloperidone during acute treatment of schizophrenia.[3] Iloperidone has also been shown to reduce the effects of apomorphine induced climbing behavior in mice as well as the effects of head twitching induced by 5-HT in rats.[4]

Possible side effects include hypotension, dizziness, and somnolence were very common side effects ranging from mild to moderate in severity. The studies indicate that repeat administration of iloperidone could decrease the effects of hypotension.[5] other side effects includes very stiff (rigid) muscles, high fever, sweating, confusion, fast or uneven heartbeats, tremors, feeling like might pass out, twitching or uncontrollable movements of eyes, lips, tongue, face, arms, or legs.

EXPERIMENTAL

Materials

Working standard of Iloperidone was obtained from well reputed research laboratories. HPLC grade Acetonitril, Methanol was purchased from E. Merck (Mumbai, India).

Apparatus

A Series HPLC system PEAK LC 7000 isocratic HPLC with PEAK 7000 delivery system. Rheodyne manual sample injector with switch (77251), Analytical column Zodiac C18. 250×4.6mm, Electronic balance-DENVER (SI234), manual Rheodyne injector with a 20 µl loop was used for the injection of sample. PEAK LC software was used. UV 2301 Spectrophotometer was used to determine the wavelength of maximum absorbance.

Determination of wavelength of maximum absorbance

The standard solutions of Iloperidone were scanned in the range of 200 - 400 nm against mobile phase as a blank. Iloperidone showed maximum absorbance at 210nm. So the wavelength selected for the determination of Iloperidone was 210nm.

Chromatographic equipment and conditions

To develop a High Pressure Liquid Chromatographic method for quantitative estimation of ILOPERIDONE an isocratic PEAK HPLC instrument with Zodiac C18 column (250 mm x 4.6 mm, 5µ) was used. The instrument is equipped with a 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.

The mobile phase consisted of Methanol: Acetonitrile 80:20v/v. Injections were carried out using a 20 µl loop at room temperature (20 + 2 °C) and the flow rate was 1 ml/min. Detection was performed at 210nm with 12min runtime.

Standard and sample solutions

A 10 mg amount of Iloperidone reference substance was accurately weighed and dissolved in 10 ml mobile phase in a 10 ml volumetric flask to obtain 1000 ppm concentrated solution. Required concentrations were prepared by serial dilution of this solution.

A composite of 20 [Fanapt -10mg] tablets was prepared by grinding them to a fine, uniform size powder. 10 mg of Iloperidone was accurately weighed and quantitatively transferred into a 100 ml volumetric flask. Approximately 25 ml mobile phase were added and the solution was sonicated for 15 min. The flask was filled to volume with mobile phase, and mixed. After filtration, an amount of the solution was diluted with mobile phase to a concentration of 60ppm.

Method validation

Method validation was performed following ICH specifications for system suitability, specificity, range of linearity, LOD, LOQ, accuracy, precision and robustness.

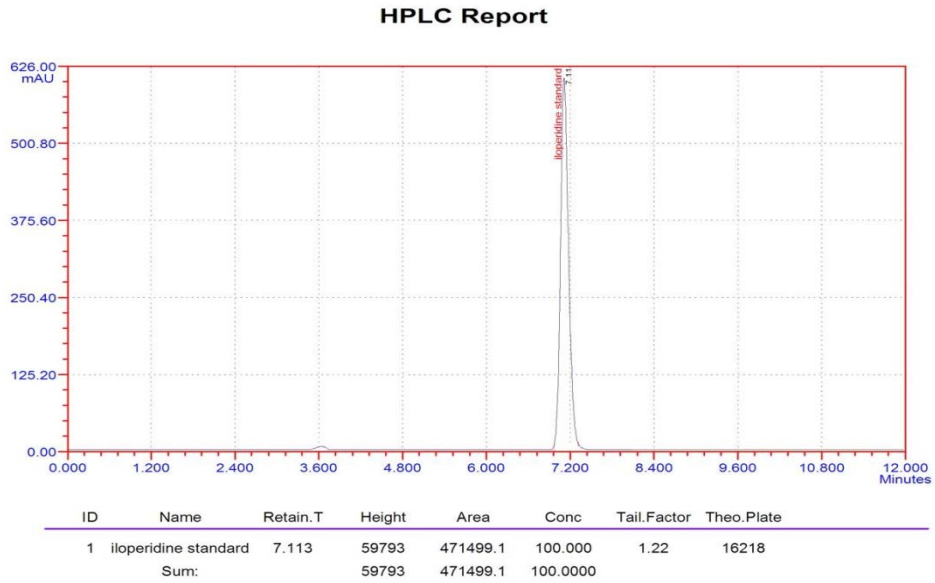
RESULTS AND DISCUSSION

System Suitability

Having optimized the efficiency of a chromatographic separation, the quality of the chromatograph was monitored by applying the following system suitability tests: capacity factor, tailing factor and theoretical plates. The system suitability method acceptance criteria set in each validation run were: capacity factor >2.0, tailing factor <2.0 and theoretical plates >2500. In all cases, the % relative standard deviation (R.S.D) for the analytic peak area for two consecutive injections was < 2.0%. A chromatogram obtained from reference substance solution is presented. System suitability parameters were shown in Table.1. Standard chromatogram was given in Figure.2.

Api Conc.	60ppm
Mobile Phase	Methanol: Acetonitrile 80:20v/v
Wavelength	210nm
Column	C_{18} Column
P^H	4.8
Retention Time	7.1min
Run Time	12min
Area	471499
Th. Plates	16218
Tailing Factor	1.22
Pump Pressure	9.0 MPa

Table.1: System suitability parameters of Iloperidone.



ID	Name	Retain.T	Height	Area	Conc	Tail.Factor	Theo.Plate
1	iloperidine standard	7.113	59793	471499.1	100.000	1.22	16218
	Sum:		59793	471499.1	100.0000		

Figure.2: Standard chromatogram of Iloperidone.

Range of linearity

Standard curves were constructed daily, for three consecutive days, using seven standard concentrations in a range of 20, 40, 60, 80, 100 and 120 for Iloperidone. The linearity of peak area responses versus concentrations was demonstrated by linear least square regression analysis. The linear regression equation was y = 7349 + 18077x (r= 0.9992). Linearity values can show in Table: 2.

S.No	Concentration (µg/ml)	Area
1	20	167526
2	40	323222
3	60	471499
4	80	612228
5	100	754358
6	120	884230
	Slope	7349
	Intercept	18077
	CC	0.999

Table.2: Linearity results of Iloperidone.

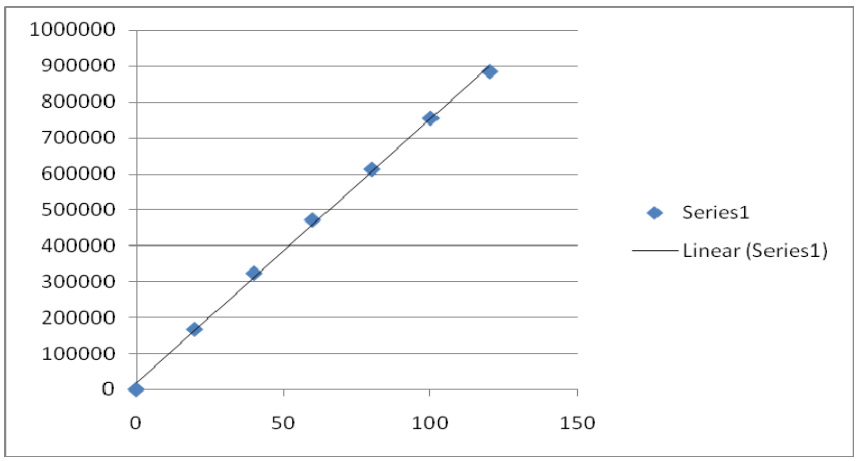


Figure 3: Calibration curve of lloperidone

Precision

To study precision, six replicate standard solutions of lloperidone (60ppm) were prepared and analyzed using the proposed method. The percent relative standard deviation (% RSD) for peak responses was calculated and it was found to be which is well within the acceptance criteria of not more than 2.0%. Results of system precision studies are shown in Table.3 and Table.4.

Sample preparation no. (API Conc. 60 µg/ml)	Area
1	475733
2	478753
3	466504
4	471830
5	476421
6	469266
RSD	0.99

Table 3: Intraday Precision Results for lloperidone.

Sample preparation no.	Area

(API Conc. 60 µg/ml)	
1	485818
2	491092
3	489670
4	484167
5	485965
6	486094
RSD	**0.54**

Table 4: Inter day Precision results of Iloperidone.

Limit of Detection and Limit of Quantification

To determine the Limit of Detection (LOD) sample was dissolved by using Mobile phase and injected until peak was disappeared. After 0.1ppm dilution Peak was not clearly observed, based on which 0.1ppm is considered as Limit of Detection and Limit of Quantification is 0.3ppm.

Parameter	**Measured Value**
Limit of Quantification	0.3ppm
Limit of Detection	0.1ppm

Table.5: LOD and LOQ results of Iloperidone.

Robustness

Typical variations in liquid chromatography conditions were used to evaluate the robustness of the assay method. The robustness study was performed by slight modification in composition of the mobile phase, pH of mobile phase and wavelength of the detector. Iloperidone at standard concentration was analyzed under these changed experimental conditions. It was observed that there were no marked changes in chromatograms, which demonstrated that the developed method was robust in nature. The robustness acceptance criteria set in the validation were the same established on system suitability test describe above. Results were shown in Table.6.

S.NO	Parameter	Change	Area	% of Change

1	Standard	No change in mobile phase preparation	473015	……
2	MP	Methanol: Acetonitrile 78:22v/v	477190	1.2
		Methanol: Acetonitrile 82:18v/v	471804	1.76
3	PH	4.6	465981	1.18
		5.0	475503	0.84
4	WL	208	476966	0.15
		212	467611	0.83

Table.6: Robustness results of Iloperidone.

Ruggedness

Ruggedness was performed by using six replicate injections of standard and sample solutions of concentrations which were prepared and analyzed by different analyst on three different. Ruggedness also expressed in terms of percentage relative standard deviation.

Sample	Conc. (ppm)	Injection No.	Peaks Area	R.S.D (Acceptance Criteria < 2.0%)
Iloperidone	60 µg/ml	1	476981	0.84
		2	474159	
		3	483243	
		4	476056	
		5	481769	
		6	483678	

Table.7: Ruggedness results of Iloperidone.

Recovery

The accuracy of the method was determined by standard addition method. A known amount of standard drug was added to the fixed amount of pre-analyzed tablet solution. Percent recovery was calculated by comparing the area before and after the addition of the standard drug. Recovery test was performed at 3 different concentrations i.e. 60ppm, 80ppm, 100ppm. The percent recovery was calculated and results are presented in Table. Satisfactory

recoveries ranging from 99.43 to 101.45 were obtained by the proposed method. This indicates that the proposed method was accurate. Results are given in Table.8.

% Recovery	Iloperidone					
	Target Conc. (ppm)	Spiked conc. (ppm)	Final Conc. (ppm)	Area	Conc., Obtained	% of Recovery
50%	40	20	60	470463	59.86	99.76
	40	20	60	475218	60.47	100.78
	40	20	60	472672	60.14	100.23
100%	40	40	80	629161	81.16	101.45
	40	40	80	614216	80.25	100.31
	40	40	80	619129	80.9	101.13
150%	40	60	100	763361	101.19	101.19
	40	60	100	760673	100.83	100.83
	40	60	100	750111	99.43	99.43

Table.8: Recovery results of Iloperidone.

Formulation	Dosage	Conc.	Amount found	% Assay
Fanapt	10mg	60ppm	59.60	99.33

Table.9: Formulation Analysis.

Degradation studies:

Forced degradation studies of both the drugs were carried out under conditions acid, alkali, peroxide, heat, Sun light, uv light, aqueous etc. After exposing sample was tested immediately and 48 hours incubation. It can be concluded that the method separates the drugs from their degradation products. It may be employed for analysis of stability samples of Iloperidone. Degradation studies are given in Table.10.

Condition after 48 hours	**Observation**
Standard	No degradation
3% Peroxide	Iloperidone degraded in to four compounds.
0.1 N Basic	Iloperidone degraded in to four compounds
0.1 N Acidic	Iloperidone degraded in to three compounds
Sun light	Iloperidone degraded in to three compounds
UV light	Iloperidone degraded in to five compounds
Aqueous (HPLC)	Iloperidone degraded in to four compounds
Thermal (heat)	Standard peak was spited into two peaks

Table.10: Degradation studies.

Stability studies

Stability test was conducted by injecting the sample solution in different time intervals after preparation. The sample has shown the stable up to 48 hours after preparation. Stability studies are given in Table.11.

S. No	**Time (hours)**	**Area**	**% of assay**
1	0	468553	99.377
2	2	471146	99.92
3	4	468711	99.4
4	6	463659	98.33
5	12	478100	101.46
6	18	473703	100.46
7	24	477160	101.2
8	36	467705	99.19
9	48	459351	97.42
10	52	414267	87.86

Table.11: Stability studies.

CONCLUSION

In the proposed study, stability-indicating HPLC method was developed for the simultaneous determination of Iloperidone and validated as per ICH guidelines. Statistical analysis proved that method was accurate, precise, and repeatable. The proposed method for the assay of Iloperidone in tablets or capsules is very simple and rapid. It should be emphasized it is isocratic and the mobile phase do not contain any buffer. The method was validated for specificity, linearity, precision, accuracy and robustness. Although the method could effectively separate the drug from its products, further studies should be performed in order to use it to evaluate the stability of pharmaceutical formulations.

REFERENCES

1. "Vanda's Schizophrenia Drug Wins Approval From U.S. Regulators" (Press release). Bloomberg. May 6, 2009. Retrieved 2009-05-06.
2. Hans O. Kalkman, Dominik Feuerbach, Erika Lötscher, Philippe Schoeffter (July 2003). "Functional characterization of the novel antipsychotic iloperidone at human D2, D3, α2C, 5-HT6, and 5-HT1A receptors". Life Sciences 93 (9): 1151–1159.
3. Scott L.Iloperidone: In Schizophrenia. CNSDrugs 2009; 23(10):867-880.
4. Szewczak MR, Corbett R, Rush DK, Wilmot CA, Conway PG, Strupczewski JT, Cornfeldt M (September 1995). "The pharmacological profile of iloperidone, a novel atypical antipsychotic agent". The Journal of Pharmacology and Experimental Therapeutics 274 (3): 1404–13.
5. Sainati SM, Hubbard JW, Chi E, Grasing K, Brecher MB (July 1995). "Safety, tolerability, and effect of food on the pharmacokinetics of iloperidone (HP 873), a potential atypical antipsychotic". Journal of Clinical Pharmacology 35 (7): 713–20.