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ESTIMATION OF 6-FLUORO-3-(PIPERIDIN-4-YL) BENZO [D] ISOXAZOLE HYDROCHLORIDE AND 1-(4-(3-CHLOROPROPOXY)-3-METHOXYPHENYL) ETHANONE OF ILOPERIDONE IN BULK AND DOSAGE FORM BY RP-HPLC

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ABSTRACT

A high performance reverse phase liquid chromatographic method was developed for the determination of related substances, 6-Fluoro-3-(piperidin-4-yl) benzo [d]isoxazole hydrochloride and 1-(4-(3-chloropropoxy)-3-methoxyphenyl)ethanone of Iloperidone in bulk and dosage form. The separation was eluted on a Zodiac C_{18} column (400 mm x 4.0 mm;5µ) using a mobile phase mixture of sodium per chlorate, acetonitrile and methanol in a gradient program at a flow rate of 0.7ml/min. The detection was made at 275 nm. The method was validated for Linearity, speficity, LOD, LOQ, accuracy, robustness, ruggedness, precision, Filter paper variation and solution stability. The retention time of Iloperidone was found to be 4.8±0.1 min. The propose method was validated as per the ICH guidelines. The method was accurate, precise, specific and rapid found to be suitable for the quantitative estimation of the impurities in drug and pharmaceutical dosage form.

KEYWORDS

Method development and validation, Iloperidone, Impurities, Zodiac C_{18} column, RP-HPLC, Tablets.

INTRODUCTION

Iloperidone is 1-[4-[3-[4-(6-Fluoro-1, 2-benzisoxazol-3-yl)-1-piperidinyl] propoxy]-3-methoxyphenyl] ethanone with a molecular formula $C_{24}H_{27}FN_2O_4$ and molecular weight 426.481. Iloperidone is an antipsychotic drug used in treatment of schizophrenia[1]. it was recently approved by the USFDA on May 6, 2009. Iloperidone is not official in any pharmacopoeia. As per the literature survey Iloperidone Determination was Development and validation of uv-spectrophotometric methods for quantitative estimation of Iloperidone in bulk and dosage form[2]. Cytochrome P450 isoforms metabolism of Iloperidone by Liquid chromatography/Mass spectrometry[3]. UV-spectrophotometric method for quantitative estimation of Iloperidone in bulk and pharmaceutical dosage form[4]. LC Method for Iloperidone in pharmaceutical Formulation[5]. Stress degradation studies on Iloperidone and development of a stability indicating HPLC method for bulk drug and pharmaceutical dosage form [6]. Genome association study identifies polymorphisms associated with QT prolongation during Iloperidone treatment of schizophrenia [7]. UV spectrophotometric and hplc methods for quantitative determination of Iloperidone in pharmaceutical dosage form [8]. Since this drug is being marketed in domestic and international market the present investigation by the author describes a rapid, accurate and precise RP – HPLC method for the determination of related substances 6-Fluoro-3-(piperidin-4-yl) benzo [d]isoxazole hydrochloride and1-(4-(3-chloropropoxy) 3 methoxyphenyl) ethanone from Iloperidone bulk sample and pharmaceutical dosage form. The detector

responses were linear in the concentration range of 2.58 – 20.64 µg/ml of drug and its related substances. The method was validated as per ICH guidelines.

EXPERIMENTAL

Chromatographic Conditions

Agilent 1200 series with high pressure liquid chromatographic instrument provided with a Zodiac C_{18} column (400 mm x 4.0 mm; 5 μ) Auto sampler, and VWD photo diode array detector, thermostatted column compartment connected with EZ Chrom software. HPLC grade methanol, acetonitrile , water were purchased from E. Merck Co; Mumbai, India, and sodium perchlorate, perchloric acid, ortho phosphoric acid AR grade were purchased from SD Fine Chem. Mumbai, India were used in the study.

Drug samples

The reference sample and impurities was supplied by Bio-Leo Analytical Labs India (P) Ltd, Hyderabad, India and tablet sample were purchased from local market.

Mobile phase

Accurately weigh 2.8g (0.02M) of sodium perchlorate was weighed out and dissolved in 1000ml of water and adjust pH 3.0 with dilute phosphoric acid. This buffer was used as mobile phase preparation A, a mixture of acetonitrile and methanol in the ratio of 80:20 v/v, for mobile preparation B, a mixture of 0.1% orthophosphoric acid in water and acetonitrile in the ratio of 50:50 v/v, used as a diluent, the solutions were filtered through 0.45µ membrane filter and was degassed and Iloperidone and its impurities were eluted in a gradient program given in **Table 1**. The mobile phase was filtered through 0.45µ membrane filter and sonicated by using Biotechnics India Sonicator, Mumbai; the flow rate of the mobile phase was maintained at 0.7ml/min. The column temperature was maintained at 30^0C and the detection of the drug was carried out at 275nm.

Table 1: Gradient Programme

Time	Mobile Phase-A	Mobile Phase-B
0.0 min	65%	35%
0.9 min	65%	35%
3.20 min	48%	52%
7.00 min	30%	70%
9.00 min	30%	70%
12.00 min	30%	70%
12.10 min	65%	35%
15.00 min	65%	35%

Diluent

Prepare a filtered and degassed mixture of 0.1% Orthophosphoric acid in water: ACN in the ratio of (50:50 v/v).

Preparation of standard stock solution

Weigh accurately 20mg of Iloperidone standard and transfers in to 200ml volumetric flask add about 30ml of diluent sonicate to dissolve resulting solution was diluted with the mobile phase.

Test preparation:

Crush the tablets in to powder weigh equivalent to 50mg of Iloperidone in 50ml volumetric flask, add 30ml of diluent and sonicate for 15 minutes with occasional shaking. Finally it is make up to

Volume with diluent.

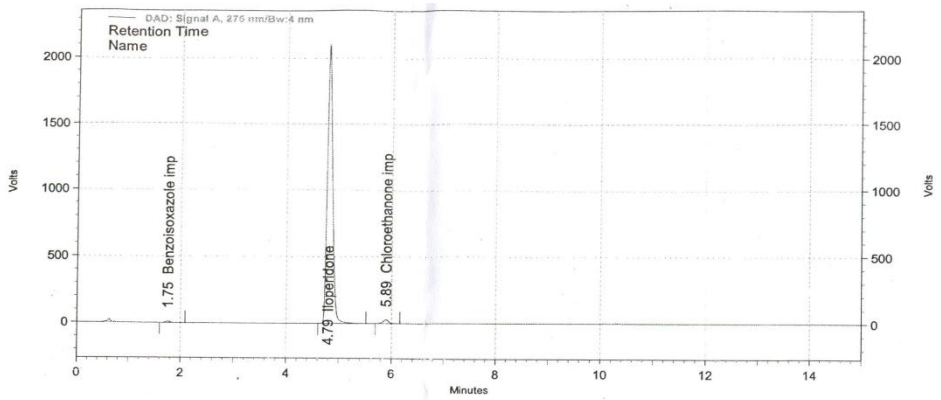
Placebo preparation:

Weigh and transfer the tablets powder equivalent to 50mg of Iloperidone in 50ml volumetric Flask, add 30 ml of diluent and sonicate for 15 minutes with occasional shaking. Finally it is make up to the Volume with diluent.

LINEARITY AND CONSTRUCTION OF CALIBRATION CURVE

The quantitative determination of the drug was accomplished by a standard method. The column was equilibrated with the mobile phase for at least 30 min prior to the injection of the drug solution. Linearity of the peak area response was determined by taking measurement at Six concentration prints (6 replicates at each point) working dilution of Iloperidone, Benzoisoxazole impurity(6-Fluoro-3-(piperidin-4-yl) benzo [d]isoxazole hydrochloride) and Chloroethanone impurity(1-(4-(3-chloropropoxy)-3-methoxyphenyl) ethanone) in the range of 2.58-20.64µg/ml, 2.56 to 20.48 µg/ml and 2.6 to 20.78 µg/ml were prepared by taking suitable aliquots of working standard solution with diluent. 5µl quantity of the dilution was injected each time in to the column at a flow rate 0.7ml/min. Each dilution was injected 6 times in to the column. The drug elutes was monitored at 275 nm and the corresponding chromatograms were obtained. Form these chromatograms the mean peak areas were calculated and a plot of concentration over the peak area was constructed. The regression of the plot was completed by least squares regression method. A linear relationship in the range was found to the 2.58-20.64 µg/ml of the drug and its impurities between the concentration of analyte, and respective peak area. This regression equation was later used to estimate the amount of Iloperidone and its impurities in pharmaceutical dosage form. A representative chromatogram for the separation of Iloperidone and its impurities is given in **Figure 1**

Fig 1: Chromatogram of Iloperidone and impurities



DAD: Signal A, 275 nm/Bw:4 nm Results

Name	Retention Time	Area	Theoretical plates (USP)	Asymmetry	Peak purity
Benzoisoxazole imp	1.75	125524	1947	1.34	1.00000
Iloperidone	4.79	30408693	10364	1.86	1.00000
Chloroethanone imp	5.89	425641	15354	1.07	1.00000
Totals		30959858			

RESULTS AND DISCUSSION

The present study was aimed at developing a sensitive precise and accurate HPLC method for the separation of Iloperidone in bulk drug and in

pharmaceutical dosage form and forced degradation. In order to achieve optimum separation of the component peaks, mixtures of buffer with methanol and acetonitrile in different combinations were tested as mobile phase on a C_{18} stationary phase. A binary mixture of buffer: methanol and acetonitrile in a gradient elution was selected as the chromatographic peaks were well defined and resolved with no tailing. The retention time obtained for Iloperidone was 4.8±0.1 for Benzoisoxazole impurity was 1.75±0.1 min and for chloroethanone impurity was 5.89±0.1min. Each of the samples was injected Six times and the Sample retention times were observed in all cases. The peak areas of Iloperidone were reproducible as indicated by low coefficient of variation. A good linear relationship (r^2 = 0.9997) was observed for Iloperidone, (r^2 = 0.9999) was observed for Benzoisoxazole impurity and (r^2 = 0.9999) was observed for chloroethanone impurity the regression characteristics are given in **Table 2**.

Table 2: Linearity of Iloperidone and impurities

	Iloperidone	Benzoisoxazole impurity	Chloroethanone impurity
Correlation coefficient	0.9997	0.9999	0.9999
Slope	290395	111534	373995
Y-Intercept	-30506.4952	-5646.7745	-16155.7454
Residual sum square	0.9993	0.9997	0.9999
Residual standard deviation	67987	16032	40062

Table 3: Recovery of Benzoisoxazole impurity

Spike level	Amount Added (ppm)	Amount Recovered (ppm)	% Recovery	% Mean Recovery
LOQ level	0.13	0.128	96.50	96.29
LOQ level	0.13	0.129	96.96	
LOQ level	0.13	0.127	95.40	
50%	5.12	4.88	95.33	96.03
50%	5.12	4.87	95.10	
50%	5.12	5.00	97.65	
100%	10.24	10.00	97.63	97.71
100%	10.24	10.03	97.91	
100%	10.24	9.99	97.59	
150%	15.36	15.25	99.29	99.19
150%	15.36	15.22	99.11	
150%	15.36	15.23	99.15	
200%	20.48	20.38	99.53	99.55
200%	20.48	20.39	99.58	
200%	20.48	20.40	99.60	

Recovery of Chloroethanone impurity

Spike level	Amount Added (ppm)	Amount Recovered (ppm)	% Recovery	% Mean Recovery
LOQ level	0.052	0.052	101.24	101.25
LOQ level	0.052	0.052	100.81	
LOQ level	0.052	0.052	101.71	
50%	5.15	5.24	101.72	101.69
50%	5.15	5.24	101.67	
50%	5.15	5.23	101.55	
100%	10.31	10.43	101.23	101.03
100%	10.31	10.39	100.82	
100%	10.31	10.49	101.81	
150%	15.46	15.77	102.05	102.06
150%	15.46	15.78	102.08	
150%	15.46	15.78	102.11	
200%	20.61	21.14	102.59	102.41
200%	20.61	21.07	102.24	
200%	20.61	21.02	102.01	

Table 4: Robustness study

Condition		% of Benzoisoxazole **impurity**	% Difference	% of hloroethanone Impurity	% Differenc e
Normal Condition (i.e as such condition)		0.0	Nil	0.0	Nil
Flow changed to 0.8ml/min		0.0	Nil	0.0	Nil
Flow changed to 0.6ml/min		0.0	Nil	0.0	Nil
Column Temperature changed to 35°C		0.0	Nil	0.0	Nil
Column Temperature changed to 25°C		0.0	Nil	0.0	Nil
Buffer pH changed to 3.2		0.0	Nil	0.0	Nil
Buffer pH changed to 2.8		0.0	Nil	0.0	Nil
Extraction time	10 min	0.0	Nil	0.0	Nil
	15 min	0.0	Nil	0.0	Nil
	20 min	0.0	Nil	0.0	Nil
Condition		% Total Impurities	% Difference	Theoretical **plates**	Tailing factor
Normal Condition (i.e as such condition)		0.10	NA	14316	1.13
Flow changed to 0.8ml/min		0.10	Nil	11793	1.12
Flow changed to 0.6ml/min		0.10	Nil	16305	1.14
Column Temperature changed to 35°C		0.10	Nil	10234	1.12

Column Temperature changed to 25°C		0.10	Nil	13594	1.11
Buffer pH changed to 3.2		0.10	Nil	13434	1.13
Buffer pH changed to 2.8		0.10	Nil	10041	1.13
Extraction time	10 min	0.10	Nil	14316	1.13
	15 min	0.10	Nil		
	20 min	0.10	Nil		

Table 5: LOD Study

S. No	Name of the Component	S/N Ratio	% level of component w.r.t to sample concentration	Value (mcg/ml)
1	Iloperidone	3.23	0.0018	1.8
2	Benzoisoxazole impurity	3.00	0.0039	3.9
3	Chloroethanone impurity	3.36	0.0015	1.5

LOQ Study

S. No	Name of the Component	S/N Ratio	% level of component w.r.t to sample concentration	Value (mcg/ml)
1	Iloperidone	10.22	0.0061	6.1
2	Benzoisoxazole impurity	10.13	0.0133	13.3
3	Chloroethanone impurity	10.02	0.0051	5.1

Table 6: Solution stability study

Time (hours)	% of Benzoisoxazole impurity	% Difference	% of Chloroethanone impurity	% Difference
Initial	0.0	NA	0.0	NA
After 24 hours	0.0	Nil	0.0	Nil
After 48 hours	0.0	Nil	0.0	Nil

Table 7: Precision study

S. No	Iloperidone	Benzoisoxazole impurity	Chloroethanone impurity
1	14808	17382	20355
2	14306	17636	19316
3	14306	17463	19209
4	14605	16359	18601
5	14564	17987	19110
6	14065	17411	20027
Avg:	14442	17373	19436
SD:	266.28	544.79	642.38
% RSD:	1.84	3.14	3.31

Table 8: Specificity study

Sl. No.	Name of the Impurity/Analyte	Peak Purity	RT (Individual)	RT (Spiked sample)
1	Iloperidone	1.00000	4.80	4.79
2	Benzoisoxazole	1.00000	1.76	1.75
3	Chloroethanone	1.00000	5.89	5.89

Table 9: Intermediate Precision (ruggedness) study

S. No	Benzoisoxazole impurity	Chloroethanone impurity
1	1.02	1.01
2	1.02	1.01
3	1.02	1.01
4	1.02	1.01
5	1.02	1.01
6	1.02	1.01
Avg:	1.02	1.01
SD:	**0.00**	**0.00**
% RSD:	**0.19**	**0.07**

Note: For Analyst – 1, Column-1 & System -1 results refer Precision

Table 10: Filter Variation Study

	Centrifuged	Nylon Filter	PVDF Filter
% of Benzoisoxazole impurity	0.0	0.0	0.0
% Difference	NA	Nil	Nil
% of Chloroethanone impurity	0.0	0.0	0.0
% Difference	NA	Nil	Nil
% of Total impurities	0.0	0.10	0.10
% Difference	NA	Nil	Nil

High recovery values obtained from the different dosage form by the proposed method indicates the method is accurate. The impurity content in capsules was quantified using the proposed analytical method are given in **Table 3**.

The percentage of individual and total impurities observed were deliberate changes in the method proves that the method is robust. The robustness study results are presented in table 4.

The lowest value of LOD and LOQ as obtained by the proposed method indicates the sensitivity of the method. The results are presented in **Table 5**. The difference between initial and bench top stability sample for % of individual impurities and total impurities were found within the acceptance criteria which indicates the solution were stable up to 48 hours. The results are presented in **Table 6**.

The precision was established by six replicate injections at LOQ level of the test preparation containing impurities of interest. The values of relative standard deviation were found to be 1.84, 3.14 and 3.31 within the acceptance limit, indicating the injection repeatability of the method. The results are presented in **Table 7**.

The specificity of the HPLC method was determined by the complete separation of impurities with Iloperidone. It was observed that there was no interference of blank and placebo at the retention time of analyte and impurity peaks. Peak purity of analyte and individual impurities should not be less than 0.99

the results of specificity data for degradation study are given in **Table 8**.

The intermediate precision (ruggedness) of the method was by carried out precision study in six preparations of a sample in a single batch sample by two different analysts, on two different columns and on two different instruments was found to be within the acceptance limit, which shows that the method is rugged. The results are presented in **Table 9**.

The filter paper variation of the method was carried out by injected filtered through different 0.45 µ membrane filters, the difference between % of individual and total impurities were found within the acceptance limit. The results are presented in **Table 10**.

Hence there is no systematic HPLC method has been developed for the estimation of related substances in Iloperidone and in pharmaceutical dosage form, the proposed method useful in regular quality control analysis in pharmaceutical formulation. The method was validated the parameters such as linearity, precision, accuracy, robustness, ruggedness, solution stability studies.

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