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Development and validation of iloperidone by UV spectrophotometric methods

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ABSTRACT

A simple, accurate and economical UV visible spectrophotometric method has been developed for evaluation of Iloperidone drug. The standard and sample solutions were prepared by using distilled water as a solvent. Quantitative determination of the drug was performed at wavelength range 220 nm. The linearity was established over the concentration range of 2, 4, 6, 8, 10, 12, 14, 16, 18, 20 µg/ml for Iloperidone with correlation coefficient value of 0.9997. Precision studies showed that % relative standard deviation was within range of acceptable limits. The mean percentage recovery was found to be 98.9%. The proposed method has been validated as per ICH guidelines. has been validated as per ICH guidelines.

Keywords: Iloperidone, UV visible Spectrophotometry, Method Validation, Antipsychotic.

INTRODUCTION

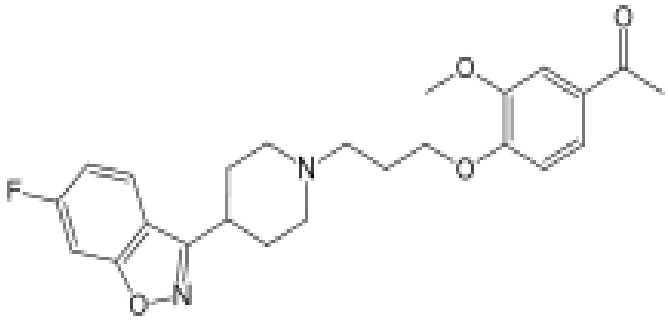
Iloperidone is an atypical antipsychotic for the treatment of schizophrenia. Iloperidone is belonging to the chemical class of piperidinyl-benzisoxazole derivatives. Till date only very few analytical methods in UV spectroscopy has been reported for the determination of iloperidone in bulk and formulations. Existing literature reveals that there are few published analytical methods for the determination of iloperidone in dosage forms.

The main objective of the present study is to validate the developed method of iloperidone by UV spectroscopy with different parameters like specificity, linearity, Precision, Accuracy, robustness, limit of detection, limit of quantification and analytical techniques for the estimation of the drug. Therefore, the present study is to develop a new, simple, rapid, efficient, reproducible and selective U.V Spectroscopic method for the determination of iloperidone in bulk and formulations.

Molecular Formula: $C_{24}H_{27}FN_2O_4$

Molecular Weight: 426.481g/mol

The Structural Formula



1-[4-[3-[4-(6-fluoro-1, 2-benzisoxazol-3-yl)-1- piperidinyl]propoxy]-3-methoxyphenyl]ethanone.

MATERIALS AND METHODS

A shimadzu1800 UV/VIS double beam spectrophotometer with 1cm matched quartz cells was used for all spectral measurements. Single Pan Electronic balance (CONTECH, CA 223, India) was used for weighing purpose. Sonication of the solutions was carried out using Ultrasonic Cleaning Bath (Spectra lab UCB 40, India) .Calibrated volumetric glassware (Borosil®) was used for the validation study. Iloperidone and methanol, chloroform Acetonitrile (Molychem, Mumbai).

METHOD DEVELOPMENT

Determination of wavelength

Based on the solubility studies of the iloperidone drug we selected the solvents like methanol, chloroform and acetonitrile of analytical grade. We dissolved the bulk drug in the three solvents and prepared the three stock solutions. Then we prepared the same concentration levels of three solutions and went for the spectrum scanning by the UV double beam spectrophotometer in between 200-400 nm wavelengths.

After careful observation of the three spectrums iloperidone has having the maximum absorbance at 228 nm for methanol as a solvent system. So we selected the analytical grade methanol as our solvent system in the estimation of Iloperidone in the bulk and dosage forms.

Preparation of the stock solution

100 mg standard Iloperidone was weighed accurately and transferred to a 100 ml volumetric flask and dissolved in methanol. The flask was shaken and volume was made up to the mark with methanol to give a solution of 1000 µg/ml. From this solution, 10 ml of solution was pipetted out and placed into 100 ml volumetric flask. The volume was made up to mark with methanol to give a solution containing 100µg/ml. Further dilutions with methanol were made from this stock solution to get required concentration.

Preparation of the sample dilutions

From the above stock solution of 100 µg/ml, further aliquots like 2, 4, 6, 8, 10, 12, 14, 16, 18, 20 µg/ml were prepared and corresponding absorbance values were determined and calculated the uv spectroscopic constants.

Sl.no	Concentration µg/ml	Absorbance -1	Absorbance -2	Absorbance -3	Average Absorbance
1	2	0.117	0.120	0.120	0.119
2	4	0.229	0.230	0.230	0.230
3	6	0.351	0.353	0.350	0.351
4	8	0.442	0.441	0.440	0.441
5	10	0.562	0.562	0.561	0.561
6	12	0.660	0.661	0.660	0.660
7	14	0.778	0.780	0.781	0.779
8	16	0.876	0.875	0.874	0.875
9	18	0.985	0.988	0.988	0.987
10	20	1.821	1.830	1.829	1.826

Determination of $A^{1\%}_{1cm}$ value

From the above abosorbances regarding with concentrations, graphs were ploted between concentration and aborbances by using least square regression equation and determined the slopes, intercepts and correlation coefficient values.

1. From concentration vs absorbance-1:
   Correlation coefficient (r^2) = 0.9997
   Intercept (A) =0.00976
   Slope (B) =0.0544

2. From concentration vs absorbance-2:
   Correlation coefficient (r^2) = 0.9997
   Intercept (A) =0.0108
   Slope (B) =0.0544
3. From concentration vs absorbance-1:
   Correlation coefficient (r^2) = 0.9997
   Intercept (A) =0.0101
   Slope (B) =0.0544

- From the above all slopes and intercepts the mean and standard deviation were calculated and listed as

Mean of slopes = 0.0544 mean of intercepts = 001022

S.D of slopes = 0 S.D of intercepts = 0.00053

- According to law of concentration

Y = m (x) + c

Y = absorbance
M = slopes average
X = concentration (10 µg/ml)
C = intercept average

- **$A^{1\%}_{1cm}$ value** is the maximum absorbance of the 1 gram/100ml solution per 1 cm path length.

Y = 0.0544 x (10) +0.01022
= 0.5542
So for 1 gram/100 ml concentration solution y = 554.2

Determination of molar absorptivity (£)

$$£ = A^{1\%}_{1cm} \times \frac{\text{molecular weight}}{10}$$

= 554.2 x 426.48/10 = 23,635.52

METHOD VALIDATION

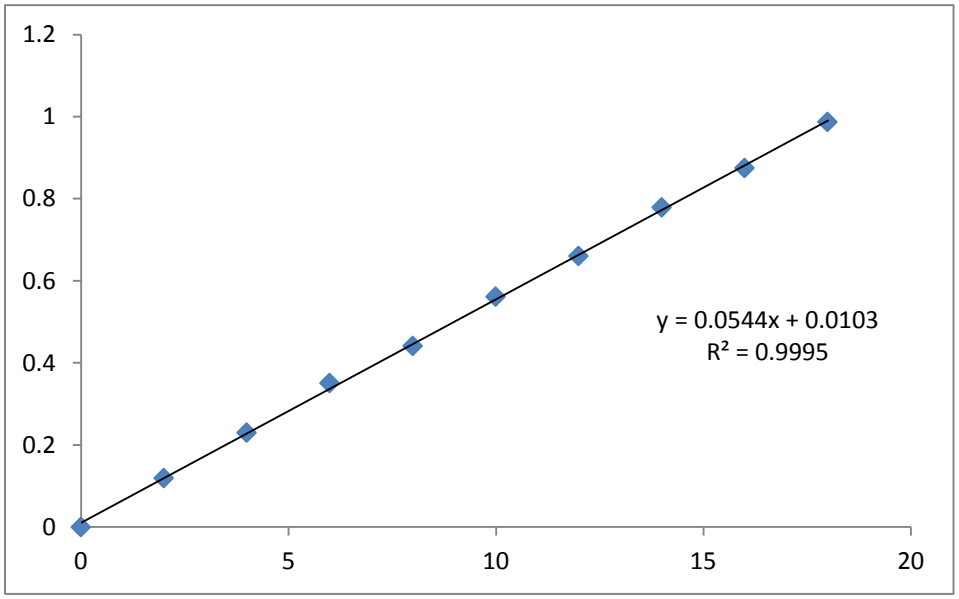
Method validation was performed in terms of linearity, precision, accuracy, limit of detection, limit of quantification, and robustness.

Linearity

The calibration curve was constructed with concentrations ranging from 2 to 18µg/ml. The absorbance of the drug was considered for plotting the graph. The linearity was evaluated by linear regression analysis, which was calculated by the least square regression method.

Sl.No	Concentration (µg/ml)	Absorbance	Statistical analysis
1	2	0.119	
2	4	0.230	Slope = 0.0544 Intercept = 0.01022 Correlation coefficient= 0.9997
3	6	0.351	
4	8	0.441	
5	10	0.561	
6	12	0.660	
7	14	0.779	
8	16	0.875	
9	18	0.987	

Calibration curve of iloperidone



The linearity was calculated by the least square regression method. A good linear relationship (r^2=0.9997) was observed between the concentrations of iloperidone and the corresponding absorbances.

Precision

To check the intra-day and inter-day variation of the method, solution containing 10 µg/ml of iloperidone was subjected to the proposed UV method of analysis. The precision of the proposed method i.e. the intra and inter-day variations in the absorbances of the drug solutions was calculated in terms of % RSD and the results are presented in the below table.

Standard concentration 10µg/ml	**Absorbance**			
	Intra day		**Inter day**	
	Analyst-I	**Analyst-II**	**Analyst-I**	**Analyst-II**
Injection.1	0.591	0.580	0.518	0.509
Injection.2	0.589	0.580	0.516	0.507
Injection.3	0.584	0.581	0.517	0.508
Injection.4	0.588	0.585	0.516	0.507
Injection.5	0.586	0.583	0.517	0.508
Injection.6	0.585	0.581	0.516	0.507
Mean	**0.587**	**0.581**	**0.516**	**0.507**
Standard deviation	**0.00263**	**0.00194**	**0.000816**	**0.00172**
% R.S.D	**0.448**	**0.33**	**0.518**	**0.339**

Acceptance criteria: %RSD of 6 replicate preparations of assay should be not more than 2%

Accuracy

The accuracy of the method was evaluated through standard addition method. In this, known amount of standard iloperidone 2 µg/ml was added in pre-analyzed sample for 5 µg/ml, 10 µg/ml and 15µg/ml. then these solutions were checked for absorbance for each spike level and the assay was performed as per the test method. From this "%Recovery" was calculated.

Accuracy data of the proposed method

Sl .No	Spiked level	Amount of drug from formulation (µg/ml)	Amount added (µg/ml)	Amount recovered (µg/ml)	%recovery	Avg & %R.S.D
1.	**50%**	5	2	A.6.87 B. 6.91 C. 6.92	98.14 98.71 98.85	98.56 0.376
2.	**100%**	10	2	A. 11.89 B. 11.90 C. 11.92	99.08 99.16 99.33	99.12 0.127
3.	**150%**	15	2	A. 16.92 B. 16.91 C. 16.89	99.52 99.47 99.35	99.44 0.087

Acceptance criteria: The mean % recovery of the iloperidone each spike level should be not less than 98.0 % and not more than 102.0 %.

Limit of detection (LOD)

The parameter LOD was determined on the basis of response and slope of the regression equation. The LOD for this method was found to be 0.0321µg/ml.

Limit of quantification (LOQ)

The parameter LOQ was determined on the basis of response and slope of the regression equation. The LOQ for this method was found to be 0.0974µg/ml.

Robustness

Robustness examines the effect of variation in operational parameters on the analysis results. For the determination of a method's robustness, parameters like variation in detector wavelength are varied within a realistic range and the quantitative influence of the variables is determined. If the influence of the parameter is within a previously specified tolerance, the parameter is said to be within the method's robustness range.

Wave Length	**227 nm**	**228 nm**	**229 nm**
Absorance-I	0.579	0.582	0.576
Absorbance-II	0.57	0.584	0.574
Absorbance-III	0.578	0.583	0.576
Mean	**0.578**	**0.583**	**0.575**
Standard deviation	**0.00057**	**0.001**	**0.0011**
% R.S.D	**0.098**	**0.171**	**0.191**

Validation Parameters

Parameter	**Result**
Absorption Maxima(nm)	228 nm
Linearity Range (µg/ml)	2-18 µg/ml
Molar Absorptivity	23,635.52

Sandell's sensitivity	0.01838
Standard Regression Equation	y=0.0544x+0.01022
Correlation Coefficient (r2)	0.9997
Accuracy(% Recovery ±Sd)	98.9±0.636
LOD (µg/ml)	0.0321 µg/ml
LOQ (µg/ml)	0.0974 µg/ml

Determination of iloperidone in tablets

The proposed method was applied to analyze commercially available dosage forms. Twenty tablets were weighed and weight equivalent to 10mg was taken in a 100 ml volumetric flask and dissolved in methanol. By Frequent shaking volume was made up to mark with methanol. A blank solution was prepared and absorbance was measured at 228 nm. The amount of iloperidone was calculated from the calibration curve. The readings were taken in triplicate and performing the same experiment for two times.

Assay of iloperidone in Tablets

Sample	Amount taken Label claim		Amount found	%assay	%R.S.D
ILOSURE TABLETS	4mg	10mg	9.792mg	97.92	0.021
	4mg	10mg	9.789mg	97.89	

Acceptance criteria for % Recovery should be between 90 to 110%. Since the %RSD of % Recovery was found to be below 2%, the assay parameter was passed.

Stability

Time (hours)	Absorbance methanol	1N HCl	0.1N HCl	1N NaOH	0.1N NaOH	6% H_2O_2
0	0.588	0.699	0.783	0.712	0.748	0.791
2	0.586	0.683	0.783	0.701	0.687	0.788
4	0.575	0.681	0.780	0.681	0.630	0.781
6	0.561	0.674	0.774	0.658	0.601	0.767
8	0.523	0.601	0.712	0.514	0.502	0.731
10	0.507	0.597	0.681	0.481	0.456	0.702
15	0.489	0.581	0.597	0.401	0.389	0.618
20	0.437	0.496	0.487	0.323	0.218	0.532
25	0.423	0.413	0.402	0.267	0.183	0.489
30	0.417	0.357	0.334	0.189	0.008	0.389
35	0.348	0.323	0.310	0.097	-	0.351
40	0.312	0.302	0.286	0.010	-	0.318
45	0.278	0.271	0.196	-	-	0.281
50	0.217	0.186	0.006	-	-	0.193
55	0.108	0.091	-	-	-	0.081
60	0.071	-	-	-	-	-

The stability of the Iloperidone was checked with time duration in methanol, acidic solution, alkaline solution and oxidizing agent solution. Acidic solution like HCl with different molarities such as 1M and 0.1M concentrated solutions were prepared and the drug content added in that

solution to attain 10 mcg/ml concentration. Alkaline solution like NaOH with different molarities such as 1M and 0.1M concentrated solutions were prepared and the drug content added in that solution to attain 10 mcg/ml concentration. Oxidising solution like 6% H_2O_2 was prepared and added the drug to attain 10 mcg/ml concentrations.

The absorbance values of these solutions were determined and compared the iloperidone drug stability in the different mediums.

Sl.no	medium	Time for 50% degradation (hrs)	Time for 100% degradation (hrs)
1	Methanol	35	60
2	1N HCl	30	55
3	0.1N HCl	25	50
4	1N NaOH	18	40
5	0.1N NaOH	15	30
6	6% H_2O_2	30	55

The stability studies of the iloperidone drug in methanol, acidic solutions (1N & 0.1N HCl), alkaline solutions (1N NaOH & 0.1N NaOH) and in oxidizing agent solution (6% H_2O_2) were checked with time duration. By this study it was found that the stability of iloperidone was more in methanol and acidic medium than the alkaline medium.

RESULTS AND DISCUSSION

- For optimize the new uv spectroscopic method for iloperidone, according to its solubility studies different solvents were tested such as methanol, chloroform and acetonitrile. Due to greater solubility and reproducible readings of maximum absorbance, methanol was taken for further work. And also the λ_{max} of the drug was found to be 228 nm.
- From the stock iloperidone solution different concentrations of 2, 4, 6, 8, 10, 12, 14, 16, 18 µg/ml were prepared and calibration curve was plotted by plotting graph between absorbance and concentration. The data was statistically validated by means of least square regression method with correlation coefficient value of 0.9997.
- The limit of detection and limit of quantization limits were calculated and these were found to be 0.0321 µg/ml and 0.0974 µg/ml respectively.
- The precision (measurements of intraday and interday) results showed good reproducibility with percent relative standard deviation (% RSD) is below 2.0. This indicates the method was precised.
- The accuracy of the method was performed by standard addition method. A standard iloperidone 2 µg/ml was added to the sample solutions of 5µg/ml, 10µg/ml and 15µg/ml and assay method was performed. The average recovery was found to be 98.56%, 99.12% and 99.44% respectively and %R.S.D was found to be 0.376, 0.127 and 0.087. The accuracy results showed good recovery with percent relative standard deviation (% RSD) is below 2.0.
- In robustness studies due to the change in wave length maxima the resulting absorbance values were showed the %R.S.D within the limit (less than 2%). This indicating that the proposed method was rousted.
- This proposed method was also applied for the assay of iloperidone in tablets. The results were showed % assay in marketed formulations like 97.90%. The results obtained were satisfactory and good agreement as per the ICH guidelines.
- The stability studies of the iloperidone drug in methanol, acidic solutions (1N & 0.1N HCl), alkaline solutions (1N NaOH & 0.1N NaOH) and in oxidizing agent solution (6% H_2O_2) were checked with time duration. By this study it was found that the stability of iloperidone was more in methanol and acidic medium than the alkaline medium.

CONCLUSION

The proposed new uv spectroscopic method for estimation of iloperidone in bulk and dosage forms was very simple, reproducible, précised, accurate and rousted one. And also this method can be successfully applied for iloperidone assay in tablet dosage forms without any interference in quality control. Analysis of the tablets by this method were reproducible, reliable and in good agreement with ICH guidelines.

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