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Validation of UV Spectrophotometric and HPLC Methods for Quantitative determination of Iloperidone in Pharmaceutical Dosage Form

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Abstract: A specific and sensitive RP-HPLC method with PDA detection and UV spectrophotometric method were developed for iloperidone in tablet dosage form. Chromatographic separation was achieved on a LiChrospher® RP-18 HPLC column (5 µ particle size and 25 cm × 4.6 mm internal diameter**)** using 0.1% trifluoroacetic acid:acetonitrile in the ratio of 50:50 v/v (pH 5.02) as mobile phase and paracetamol as the internal standard. The effluent was monitored at 275 nm. Two sharp peaks were obtained for internal standard and iloperidone at 2.8 and 7.6 min, respectively. UV spectrophotometric method was performed at 229 nm using methanol as the solvent. Linear range was 1-10 µg mL^{-1} (r^2=0.9986±0.0009) for HPLC method and 2-20 µg $^{-1}$ (r^2=0.9988±0.0004) for UV spectrophotometric method. Validation as per ICH guidelines and statistical analysis showed that both the methods were precise, accurate, sensitive, and can be used for the routine quality control of iloperidone in pharmaceutical dosage forms.
Key words: Iloperidone, RP-HPLC, UV-spectrophotometric, internal standard, ICH guidelines.

INTRODUCTION

Iloperidone is an antipsychotic drug used in the treatment of schizophrenia. Chemically it is designated as 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 426.48[1]. A thorough literature survey revealed LC-MS method for the determination of iloperidone in human plasma by Mutlib and Strupczewski[2] and application of LC-MS to identify the human liver cytochrome P450 isoforms involved in the metabolism of iloperidone by Mutlib and Klein[3]. Mandpe and Varsha has reported stress degradation behaviour of iloperidone and a stability indicating assay method by RP-HPLC[4]. However, RP-HPLC method employing internal standard and UV spectrophotometric method for the quantification of iloperidone in pharmaceutical dosage form has not yet reported in the literature. Internal standard methods are preferred for quality control of drugs since it overcomes errors from sample preparation and run to run variations, thus improving the accuracy and precision of the method. Moreover, these methods are highly sensitive and specific[5]. UV spectrophotometric method is rapid, very simple, economic, and it allows the quantification in pharmaceuticals with satisfactory reliability[6].

Therefore the aim of the present study was development and validation of RP-HPLC method using a suitable internal standard and UV-spectrophotometric method for the estimation of iloperidone from its tablet dosage form.

MATERIALS AND METHODS

Analytically pure sample of iloperidone and paracetamole (assigned purity >99%purity) were obtained as gift samples from Sun Pharmaceuticals Ltd, Chennai. HPLC grade methanol was obtained from Merck specialties Pvt. Ltd. (Mumbai, India). Water for RP-HPLC was prepared using Millipore purification system (Milli-Q, Bangalore, India) and double distilled water was used to prepare all the solutions for UV spectrophotometric experiments. Tablet formulation (Ilosure, Sun Pharmaceuticals Ltd, Chennai) containing 2 mg iloperidone was purchased from the local pharmacy.

Instrumentation and analytical conditions

Chromatography was performed under ambient conditions with liquid chromatograph mass spectrometer (Shimadzu LCMS-2010EV) equipment comprising of a binary gradient pump (LC-20AD), degasser (DGU-20A_3), and a variable wavelength programmable PDA detector (SPD-M20A) with auto sampler system (SIL-20AC). The instrumentation was controlled by Shimadzu LCMS Solution software. Chromatographic separation was achieved on a LiChrospher® RP-18 HPLC column (5 µ particle size and 25 cm × 4.6 mm internal diameter) using the mobile phase composed of 0.1% trifluoroacetic acid : acetonitrile in the ratio of 50:50 v/v (pH 5.02; adjusted with 1% triethylamine) at a flow rate of 1 ml/min. The mobile phase was filtered through 0.45 µ nylon filter and degassed by sonication prior to use. Column temperature was maintained at ambient and the run time was set at 10 min. The column was equilibrated for 30-40 min with mobile phase prior to injection of the analyte. The volume of injection was 20µL. Detection was performed at 275 nm since both the components showed reasonable absorbance at this wavelength. The UV spectrophotometric method was performed using a Jasco V-630 UV/VIS Spectrophotometer with Spectra Manager (Version 2) controlling software at 229 nm in 1 cm quartz cells. The samples were prepared using methanol.

Preparation of standard solutions

For HPLC method, 100µg mL^{-1} of iloperidone and paracetamol were prepared separately by dissolving 10 mg each in 100 ml methanol. Working standard solutions of iloperidone (1 - 10 µg mL^{-1}) were prepared by transferring 0.1 to 1 ml of iloperidone stock solution to serially arranged 10 ml standard flasks and diluting to volume using the mobile phase. A constant volume (0.2 ml) of stock solution containing paracetamol was added as the internal standard before making the dilutions.

For the UV spectrophotometric method, stock solution of 100 µg mL^{-1} of iloperidone was prepared in methanol. The working standard solutions were prepared by dilution of the stock solution with methanol to get a concentration range 2 - 20 µg mL^{-1}. The absorbance was measured at 229 nm using methanol as blank.

Preparation of the sample solutions

HPLC method

Twenty tablets (Ilosure) were weighed, powdered and a powder mass equivalent to 2 mg of iloperidone was transferred to a 50 ml volumetric flask containing methanol. The mixture was then sonicated for 20 min to dissolve the material completely and centrifuged at 3,000 rpm for 5 min. An aliquot of supernatant solution (1.25 ml) was transferred to 10 ml volumetric flask, fixed concentration of internal standard (0.2 ml) was added and diluted to 10 ml using the mobile phase to get a solution of 5µg mL^{-1} of iloperidone and 2 µg mL^{-1} of IS. The resulting solution was analyzed by the proposed method. All determinations were carried out in six replicates.

UV Spectrophotometric method

Twenty tablets (T.Ilosure), each containing 2 mg of iloperidone was weighed and finely powdered. A powder mass equivalent to 2 mg of iloperidone was transferred to 50 ml volumetric flask, dissolved with methanol and the volume was made up to get a concentration of 40 µg mL^{-1}. The mixture was then sonicated for 20 min to dissolve the material completely and centrifuged at 3,000 rpm for 5 min. An aliquot of supernatant solution (2.5 ml) was transferred to 10 ml volumetric flask and made upto volume using methanol. The resulting solutions were then analyzed spectrophotometrically at 229 nm.

Method Validation

Both the methods were validated by following ICH recommendations for validation of analytical procedures[7].

Linearity and range

For the HPLC method, stock solution of iloperidone was suitably diluted with the mobile phase to get concentrations in the linear range of 1 - 10 µg mL^{-1}. Then 2 µg mL^{-1} of paracetamol (IS) was added to each dilution and 20 µl was injected into the column, the peak area and retention times were recorded. The calibration curve for iloperidone was constructed by

plotting the ratio of the peak area of iloperidone to that of internal standard (Y) against concentration (X) and linearity was evaluated by linear regression equation. The slope, intercept and correlation coefficient values were recorded. For the UV Spectrophotometric method, calibration graph was prepared with 2 - 20 µg mL^{-1} of iloperidone and absorbance was recorded at 229 nm. Each experiment was performed in six replicates.

Accuracy

Accuracy was determined by standard addition method. To a pre-analyzed sample formulation a known quantity of standard was added at three levels (50, 100 and 150% of the assay concentration). A constant concentration (2 µg mL^{-1}) of IS was added to all the samples in HPLC experiment and the mixtures were analyzed by the developed method. Absorbance was measured directly for the UV spectrophotometric method. The experiment was performed in six replicates. %RSD and %recovery were calculated for all the concentrations.

Precision

The precision of the method was studied by repeatability (within-day) and intermediate precision (inter-day).The intra-day precision studies were carried out by estimating the response six times on the same day using three different concentrations (4, 5 and 6 µg mL^{-1} for HPLC method and 6, 10 and 14 µg mL^{-1} for UV spectrophotometric method) of iloperidone and inter-day precision studies were done by repeating the above procedure on three different days. A fixed concentration (2 µg mL^{-1}) of the internal standard was added to all the samples in HPLC experiment. The results of precision studies were expressed as %RSD.

LOD and LOQ

Sensitivity of HPLC and UV methods were determined from limit of detection (LOD) and limit of quantitation (LOQ). The LOD and LOQ were calculated from the calibration curve using the following equations

LOD = 3.3xσ/S and

LOQ = 10 x σ/S, where,

σ = standard deviation of y intercept of regression line

S = slope of the calibration curve

Specificity

Purity of iloperidone and IS peaks were assessed in HPLC method by comparing the individual spectrum at three regions i.e. peak start, peak apex and peak end. The specificity of the method was further assessed by comparing the chromatograms obtained from standards and from placebo solutions prepared using the excipients most commonly present in pharmaceutical formulations, including lactose monohydrate, starch, hydroxypropyl methyl cellulose, and magnesium stearate. Specificity of UV spectrophotometric method was determined from the absorption spectra of iloperidone reference standard and that of formulation.

Robustness

Robustness of HPLC method was studied to evaluate the effect of small but deliberate variations of the chromatographic conditions on the method parameters. Robustness was determined by changing individually the flow rate (1±0.1ml/min), organic solvent (50±1%), pH (5.02±0.01) and evaluating their effects on peak parameters.

System suitability tests

The test was carried out by making six replicate injections of a standard solution containing 5 and 2 µg mL^{-1} of iloperidone and IS, respectively. The peak area of the sample and IS , number of theoretical plates (N), resolution(R_s), tailing factor (T),capacity factor (k'), and asymmetric factor (A_s) were analysed.

Stability Studies

A suitably diluted solution of iloperidone and IS in methanol was stored under laboratory bench conditions upto 48 hours, and under refrigeration (8±0.5ºC) for 5 days. The solutions were assayed by the proposed method.

Figure 1: Overlay spectrum of iloperidone and internal standard[Paracetamol]

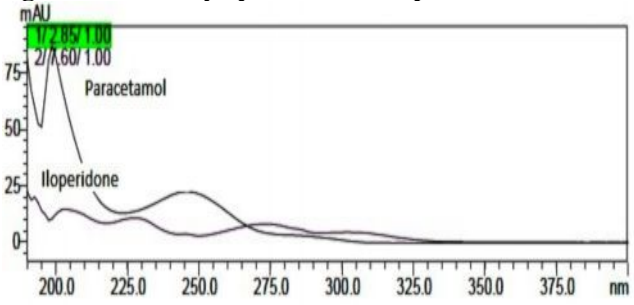


Figure 2: RP-HPLC chromatogram of standard solution of iloperidone (5 µg mL^{-1}) with internal standard (2 µg mL^{-1}) at 275 nm

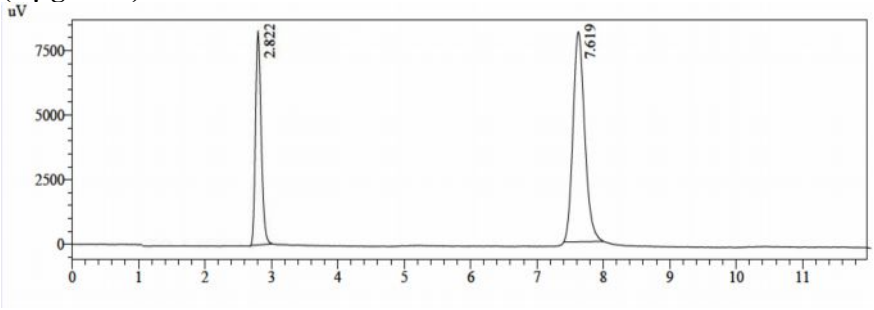


Table 1: Regression parameters and validation parameters of iloperidone by RP-HPLC and UV-spectrophotometric method

Parameter	RP-HPLC method	UV-Spectrophotometric method
Concentration range	1-10	2-20
Slope[a]	0.3338±0.01966	0.05535 ±0.00010
Intercept[a]	-0.1138±0.01743	-0.01602 ±0.00031
Correlation coefficient (r^2)	0.9986±0.0009	0.9988± 0.00040
LOD (µg mL^{-1})	0.1766 µg mL^{-1}	0.0185 µg mL^{-1}
LOQ (µg mL^{-1})	0.5889 µg mL^{-1}	0.0562 µg mL^{-1}
Recovery (%)[a]	100.193 ±0.4726	100.048±0.1352
Intra-day precision (RSD, %)[a]	0.35-0.60	0.04-09
Inter-day precision (RSD, %)[a]	0.37-1.14	0.07-0.14

[a] Mean ± SD, n = 6

Table 2: Assay of iloperidone in formulation by RP-HPLC and UV-spectrophotometric method

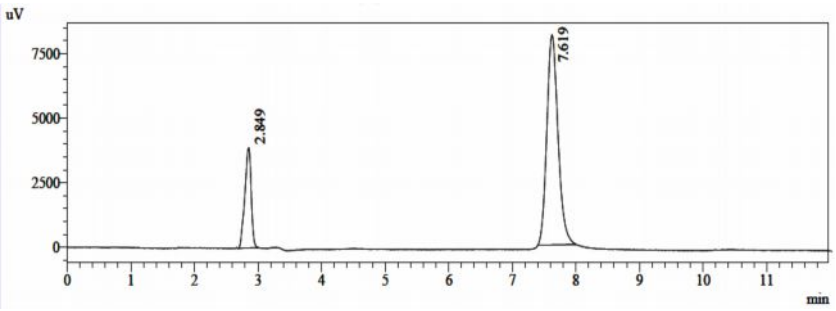
Method	Labeled amount (mg/tab)	Amount found (mg)[a]	Assay (%)	RSD (%)
RP-HPLC method	2	1.9977±0.0042	99.9133	0.2579
UV Spectrophotometric method	2	2.0015±0.0019	100.0566	0.12712

[a] Mean ± SD, n = 6

Table 3: System suitability parameters of the proposed RP-HPLC method

Parameter	Peak	
	Drug	IS
Retention time (t_R)	7.619	2.849
Peak purity index	1	0.999
Asymmetry	1.120	0.99
Tailing factor *(T)*	1.245	0.97
Capacity factor (*k'*)	18.05	6.122
Theoretical plates (*N*)	8620	3140
Selectivity (α)	4.77	-
Resolution (R_s)	8.5	-

Figure 3: RP-HPLC chromatogram of formulation of iloperidone (5 µg mL^{-1}) with internal standard (2 µg mL^{-1}) at 275 nm



RESULTS AND DISCUSSION

HPLC Method: The current study describes a new RP-HPLC method and UV-spectrophotometric method for the determination of iloperidone in bulk and tablet dosage form. The retention times of IS and iloperidone was 2.8 and 7.6 min, respectively. Figure 1 shows the overlay spectrum of IS and iloperidone. The typical chromatograms of iloperidone with internal standard are shown in figure 2.

The developed method was validated in terms of specificity, linearity and range, accuracy, intra-day and inter-day precision, LOD, LOQ, robustness and system suitability as per the recommendations of ICH guidelines. Specificity of the method was established by the complete separation of the peaks of iloperidone and internal standard in the presence of tablet excipients and their peak purity index which was found to be 1. The standard calibration curve was linear over the concentration range 1 to 10 µg mL^{-1} with a correlation coefficient (r^2) 0.9986, the corresponding linear regression equation being y = 0.3338x-0.11386. LOD and LOQ were found to be 0.1766 µg mL^{-1} and 0.5889 µg mL^{-1} respectively, indicating the sensitivity of the method. Recovery experiments demonstrated satisfactory accuracy with small relative standard deviations (RSD %). The mean recovery ranged from 99.92±0.3553 to 100.61±0.2579%. The developed RP-HPLC method was precise since the %RSD values were <2 for both repeatability and intermediate precision studies as recommended by ICH guidelines. RSD of repeatability (intra-day) and intermediate precision (inter-day) ranged from 0.3521 to 0.6085 and 0.3756 to 1.1486 respectively. **Table 1** illustrates the regression parameters and validation parameters of iloperidone by RP-HPLC method.

Deliberate changes in experimental conditions did not alter peak symmetry and there was no significant change in the retention time of IS and iloperidone during these experiments. The %RSD for each method parameter was calculated and was found to be <2. Present chromatographic conditions established the assay of commercial tablet of iloperidone and the results were in good agreement with the labelled claim, indicating the suitability of the method. The percentage amount of iloperidone present in the commercial formulation (T.Ilosure) was found to be 99.9133±0.2579 (table 2). Excipients present in the tablet did not interfere with the analyte peaks. Figure 3 shows the separation of analyte in the formulation and IS. Adequacy of the proposed RP-HPLC method for routine analysis of iloperidone was assured by system suitability tests. The capacity factor (*k'*) of IS and drug were found to be 6.122 and 18.05 respectively, signifying that both the peaks were well resolved with respect to the void volume. The tailing factor of 0.97 and 1.2 for IS and iloperidone peaks reflect good peak symmetry. The resolution (*Rs*) for the principle peak and internal standard was found to be 8.5, showing good separation. The theoretical plate number (*N*) was found to be 8620 for drug, thus demonstrating good column efficiency. The result of system suitability tests (table 3) shows that the newly developed method fulfils these requirements within the accepted limits. Solution stability studies demonstrated good stability of standard solution and sample solutions during all storage conditions and confirmed the applicability of the method for routine analysis.

UV Spectrophotometric method: The solutions were scanned in the wavelength range of 200-400 nm after making suitable dilutions from the stock solutions. Iloperidone showed absorption maxima at 229 nm. The method was validated as per ICH guidelines. Iloperidone in methanol showed linear relationship in the concentration range of 2 to 20 µg mL^{-1} with a correlation coefficient (r^2) 0.9988±0.0004. The regression equation was found to be Y = 0.05537-0.01595. The recoveries of iloperidone estimated by standard addition method for the three concentrations ranged from 99.77 to 100.04% with acceptable %RSD values. LOD and LOQ were found to be 0.0185 µg mL^{-1} and 0.0561 µg mL^{-1} respectively. The % RSD values for intra-day and inter-day precision varied from 0.0417 to 0.0833 and 0.0793 to 0.1485 (table 1) respectively. The amount assayed was in good agreement with the label claim (table 2) with RSD values less than 2%.

Chromatographic conditions were carefully optimized to get satisfactory resolution between analyte and IS.

The final decision on mobile phase composition and flow rate was made on the basis of peak shape (peak area, asymmetry, tailing factor), baseline drift, time required for analysis, and cost of solvents. The optimized mobile phase was 0.1% trifluoroacetic acid:acetonitrile (pH 5.02) in the ratio of 50:50 v/v. Initial trial with water:methanol (20:80 v/v) produced no peak for iloperidone. Subsequently, 0.1% TFA:methanol (50:50 v/v) showed tailing factor 2.4 while 0.1% TFA:acetonitrile (50:50 v/v) gave a broad asymmetric peak at 9 minutes. Peak shape was found to improve when the pH was increased to 4. At pH 5.02, a good symmetric peak was obtained at 7.6 minutes. Resolution (8.5) between the analyte and IS was also satisfactory using this mobile phase. The value of resolution meets the acceptance criteria which should be greater than or equal to 2^5. Various IS like aceclofenac, diclofenac, paracetamol and ibuprofen were tried. Among these, paracetamol was chosen due to less run time (2.8 min), good peak shape, adequate resolution and easy availability. Diclofenac showed retention time greater than 19 minutes. The correlation between concentration and response was also excellent in both the established methods. The retention time observed at 2.8 and 7.6 min for drug and IS allowed rapid quantification of iloperidone which is important in routine analysis. The proposed methods were also found to be precise and accurate, as depicted by the statistical data of analysis. Recovery values met the acceptance criteria of 100±2% at the three different concentration levels evaluated. The current UV spectrophotometric method allowed rapid quantification of iloperidone in tablets. Methanol was chosen as the solvent because of good solubility and stability. The spectral characteristics were also good in this solvent. Though iloperidone showed two absorption maxima at 229 and 275 nm, 229 nm was selected as the detection wavelength since it showed better linearity and sensitivity at this wavelength with minimal interferences. Low values of %RSD signify the precision of the method. A good accuracy of the method was documented with mean recoveries close to 100%. Moreover, the method is highly sensitive as evidenced by the LOD and LOQ values.

CONCLUSIONS

The validated HPLC and UV methods were found to be accurate, precise and reliable. UV spectrophotometric method was simpler and sensitive than HPLC method and the same may be used as an alternative method when advanced instruments like HPLC are not available for routine quantification purpose. Both the methods can be employed for the routine quality control of iloperidone in tablet dosage forms.

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