DEVELOPMENT AND VALIDATION OF A RAPID RP-HPLC METHOD FOR THE ESTIMATION OF ZIPRASIDONE HYDROCHLORIDE MONOHYDRATE IN BULK AND ITS CAPSULE DOSAGE FORMS.

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ABSTRACT

A rapid and sensitive Reverse Phase High Performance Liquid Chromatographic [RP-HPLC] method was developed for the estimation of Ziprasidone HCl Monohydrate [ZHM] in pure and its capsule dosage forms. The method was validated as per International Conference on Harmonization [ICH] guidelines. YMC C_{18} column (150×4.6mm, 3µm) was used with a mobile phase containing a mixture of phosphate buffer (pH-3) and methanol in the ratio of 60:40% v/v. The analysis was performed with run time of 5 minutes at a flow rate of 1ml/min. The effluents were monitored at 219nm with UV detection and ZHM was eluted at 2.750min. The method was linear (r^2 = 0.9999) at concentration ranging from 10 to 50µg/ml, precise (intra-day relative standard deviation [RSD] and inter-day RSD values < 1.0%), accurate (mean recovery = 100.08%), specific and robust. Detection and quantification limits were 0.002 and 0.007µg/ml, respectively. The results showed that the proposed method is suitable for the precise, accurate and rapid determination of ZHM in bulk, its capsule dosage forms and dissolution samples of capsules.

Keywords: Ziprasidone, RP-HPLC, Validation, RSD, Capsule.

INTRODUCTION

Ziprasidone Hydrochloride Monohydrate [ZHM] is an atypical antipsychotic drug that is chemically unrelated to phenothiazine or butyrophenone antipsychotic agents. It is a benz-isothiazoyl-piperazine derivative and it is used in the treatment of schizophrenia, mania and mixed states associated with bipolar disorder. Ziprasidone HCl has a potent selective antagonist activity for the serotonin Type 2 ($5HT_2$), dopamine Type 2 (D_2), 1 and 2 adrenergic, and H_1 histaminergic receptors[1].

ZHM is chemically known as 5-[2-[4-(1,2-benzothiazol-3-yl)piperazin-1-yl]ethyl]-6-chloro-1,3-dihydroindol-2-one hydrochloride [Figure 1].

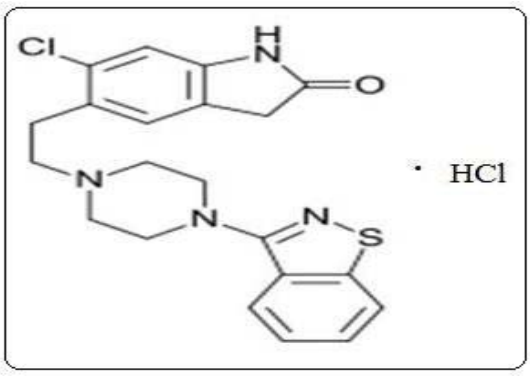


Figure-1: Chemical Structure of ZHM

Comprehensive literature survey reveals that several analytical methods have been reported for the estimation of ZHM which includes Reverse Phase High Performance Liquid Chromatographic [RP-HPLC], High Performance Thin Layer Chromatography [HPTLC], UV-Visible Spectrophotometry and Electrophoresis. Most of the analytical methods carried out by RP-HPLC to determine ZHM found in the consulted literature, are aimed at quantifying Ziprasidone in biological fluids includes RP-HPLC using UV[2] and Fluorescence[3] detection as well as LC-MS/MS[4] method. Other methods reported to determine ZHM in bulk and its pharmaceutical dosage forms includes RP-HPLC[5,6], Normal and Reverse phase HPTLC[7], Capillary Zone Electrophoresis[8], UV- Spectrophotometric[9,10] and Visible-Spectrophotometric[11,12] methods.

The target of this study is to develop a new, simple and fast analytical method by RP-HPLC to quantify ZHM in bulk and its capsule dosage forms together with its latter validation study. This validation study is defined as the laboratory studies by which it is established that the performance characteristics of the method meet requirements for the intended analytical application.

This work describes the validation parameters stated by the International Conference on Harmonization [ICH] guidelines[13,14] includes specificity, linearity, range, accuracy, precision, robustness to achieve an analytical method with acceptable characteristics of suitability, reliability and feasibility.

MATERIALS AND METHODS

Instrumentation

Quantitative HPLC was performed on a High performance liquid chromatography equipped Waters-2695 separation module with Auto Sampler and Waters-2487 dual λ-absorbance UV detector. The data processing was performed using EM-power software.

Standards and chemicals

Pure ZHM obtained as a gift sample from Watson Pharma Pvt. Ltd., Mumbai. Commercial capsule formulations Zipsydon (Sun Pharma) and Geodon (Pfizer) were used for present study containing 20mg and 80mg of ZHM,

respectively. HPLC grade methanol and water as well as Disodium hydrogen phosphate anhydrous, A.R. grade were purchased from Merck, Mumbai, India. All other chemicals used were of HPLC grade or A.R. grade.

Chromatographic conditions

The mobile phase used in this study was a mixture of methanol and phosphate buffer (pH-3) in the ratio of 60:40% v/v. Stationary phase was YMC C_{18} reverse phase column (150×4.6mm, 3µm) dimensions at ambient temperature. The contents of the mobile phase were filtered before use through a 0.45µ membrane. The mobile phase was pumped from the solvent reservoirs to the column at a flow rate of 1ml/min for 5min. The elute was monitored at 219nm using UV-detector. The retention time of the drug was found to be 2.750min.

Preparation of standard drug solutions

The standard stock solution of the ZHM was prepared by dissolving 100mg of pure ZHM in 100ml of mobile phase to give the final concentration of 1000µg/ml. The solution was sonicated for 5 minutes to insure the dissolution of ZHM. The working standard solution of ZHM was prepared by taking suitable aliquots of drug solution from the standard stock solution and the volume was made up to 10 ml with mobile phase to get concentrations of 10 to 50µg/ml. The solutions were filtered through 0.45µ membrane filter before injection and 20µl solution was injected in six replicates to the chromatographic system.

Preparation of sample solutions

For the preparation of sample solutions, twenty capsules were weighed, powder was collected and mixed. A quantity equivalent to 20 mg of ZHM was transferred into extraction flask, to this suitable amount of mobile phase was added and the mixture was subjected to sonication for 30 min for complete extraction of drug. The solution was filtered into 100ml volumetric flask and made up to the mark with mobile phase. From this, different aliquots were taken in separate 10ml volumetric flasks. The contents of the flasks were made up to the volume with mobile phase to get 20µg/ml concentration and mixed well. The solutions were filtered through 0.45µ membrane filter before injection and 20µl solution was injected in six replicates to the chromatographic system.

Method validation

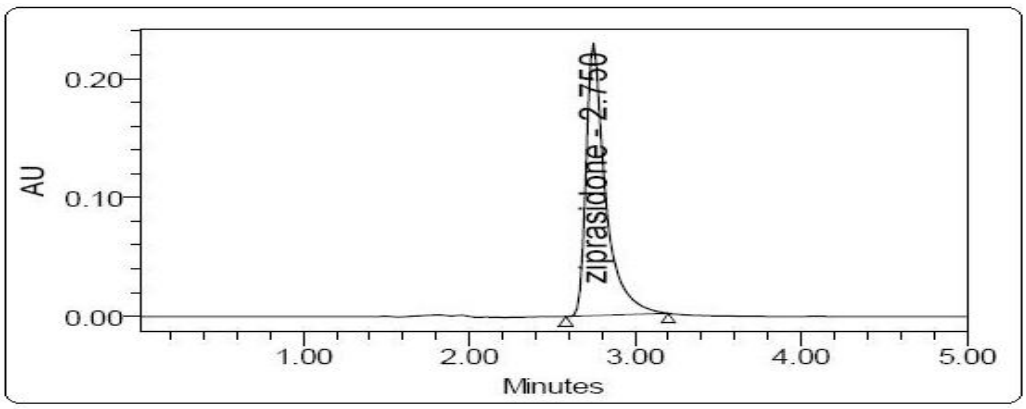
The objective of method validation is to demonstrate that the method is suitable for its intended purpose as it is stated in ICH guidelines Q2A and Q2B. Recommend validation characteristics depend on the type of analytical procedure. An important part of method validation is system suitability test. We have established a number of theoretical plates, peak asymmetry, and repeatability of injection (retention times and peak areas were checked). Method validation characteristics were tested in accordance with ICH guidelines. Method specificity was verified by comparing the chromatograms of sample of pharmaceutical preparation, standard solution and placebo (mixture of the drug-excipients excluding API). Linearity (correlation coefficient) was tested in the range 10–50µg/ml. Intra and inter-day instrumental system precision as well as repeatability and intermediate method precision was obtained as %Relative Standard Deviation [% RSD] using six replicates per day. We have established method accuracy (%Recovery and %RSD) by spiked placebo recovery method. Limits of detection and quantification were provided for ZHM. Calculation was made by means of signal-to-noise ratio method. Also, method robustness with regard to change in mobile-phase composition (±10%) and change in flow rate (±20%) was studied.

RESULTS AND DISCUSSION

HPLC method development and optimization

The chromatographic method was optimized by changing various parameters, such as the mobile phase composition, pH of the buffer used in the mobile phase. Retention time and separation of peak of ZHM were dependent on pH of the buffer and the percentage of methanol. Different mobile phases were tried, but satisfactory separation and good symmetrical peak were obtained with the mobile phases consisting of methanol and phosphate buffer (pH-3) in the ratio of 60:40% v/v. A typical chromatogram obtained by using the aforementioned mobile phase and 20µl of the injected assay preparation is illustrated in figure-2.

Figure 2: A typical chromatogram showing the peak of ZHM



Method validation

The analytical method was validated as per ICH guidelines with respect to parameters such as linearity, precision, accuracy, specificity, limit of quantification [LOQ], limit of detection [LOD] and robustness.

System suitability

For system suitability, six replicates of standard solution were injected and studied the parameters like theoretical plates, theoretical plates per meter, tailing factor (k) and Height Equivalent Theoretical Plate. The represented data was shown in table-1.

Table 1: System suitability parameters for ZHM by proposed method

Parameters	Values
Theoretical Plates (n)	3494
Theoretical plates per meter (N)	23293.33
Height equivalent to theoretical plates [HETP] (mm)	0.0429
Tailing factor (k)	1.65

Specificity

The HPLC chromatograms recorded for the placebo showed almost no peaks within a retention time range of 5min.The peak for ZHM is clearly separated from other excipients of the formulations. The retention time, tailing factor and peak area of the ZHM in marketed formulations and in dissolution samples of capsules were not affected with excipients present in formulations as well as with capsule cells, indicating a high degree of specificity of this method. Thus, the HPLC method presented in this study is specific for ZHM.

Linearity

The standard curve was obtained in the concentration range of 10-50µg/ml. The linearity of this method was evaluated by linear regression analysis. Slope, intercept and correlation coefficient [r^2] of standard curve were calculated and given in figure-3 to demonstrate the linearity of the method.

Precision

In the study of the instrumental system precision where, a RSD of 0.232% was obtained for retention time, and of 0.335% for the area obtained corresponding to the first day, being 0.112% and 0.091% for the second day, respectively (with *n* = 6 number of analyses per day).

The inter-day study (*n* = 18 analyses) carried out showed a RSD of 0.484% for retention time and 0.356% for the area obtained. In all these cases the RSD obtained was far below 1%, the limit percentage set for the precision study of the instrumental system, thus showing that the equipment used for the study worked correctly for the developed analytical method, and being highly repetitive.

The method precision study for standard solutions (*n* = 6 analyses) showed a RSD of 0.300% for the area obtained which shows good repeatability of the method.

For the intermediate precision, a study carried out by the same analyst working on different days (*n* = 6 number of analyses per day). The results calculated as inter-day RSD corresponded to 0.433%. The same study was carried out for different analysts (*n* = 6 number of samples per analyst) obtaining a RSD of 0.973%. Both results together with the individual results are showing that the proposed analytical technique has a good intermediate precision.

Accuracy

The accuracy of the method was determined on three concentration levels by recovery experiments. The recovery studies were carried out six times by spiked placebo recovery method and the percentage recoveries with standard deviations [SD] were calculated. From the data obtained which given in table-2 the method was found to be sufficiently accurate.

Figure 3: Calibration curve for ZHM

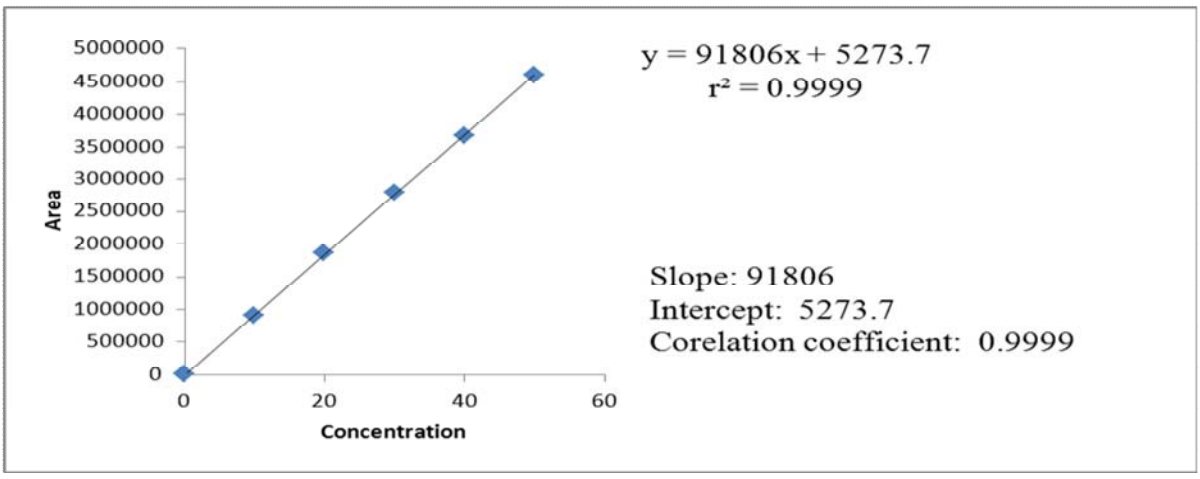


Table 2: Recovery studies for ZHM by proposed method

Amount of pure ZHM added to the placebo in µg/ml	Amount of ZHM found in µg/ml (Mean ± SD)	% Recovery of ZHM (Mean ± SD)	% RSD
10 (50%)	9.854 ± 0.007	98.841 ± 0.071	0.072%
20 (100%)	20.056 ± 0.027	100.278 ± 0.133	0.133%
30 (150%)	30.243 ± 0.038	101.114 ± 0.127	0.126%

Table 3: Assay results for capsules

Capsule formulation	Label claim	Amount of ZHM found (Mean ± SD)	% Recovery (Mean ± SD)	%RSD
Zipsydon (*Sun*)	20 mg	20.064 ± 0.025	100.322 ± 0.124	0.124%
Geodon (*Pfizer*)	80 mg	80.222 ± 0.096	100.278 ± 0.120	0.120%

LOD and LOQ

Limit of detection was found to be 0.002µg/ml (signal to noise ratio is 3) and Limit of quantification was found to be 0.007µg/ml (signal to noise ratio is 10).

Robustness

The percentage recovery of ZHM was good under most conditions and didn't show any significant change when the critical parameters were modified. The tailing factor for ZHM was always less than 2.0 and it was well separated under all the changes carried out. Considering the modifications in the system suitability parameters and the specificity of the method it can conclude that the method is robust.

Assay of the capsules

The method is sensitive and specific for the quantitative determination of ZHM and also validated for different parameters, hence has been applied for the estimation of drug in capsule dosage forms. Capsules from two different manufacturers were evaluated for the amount of ZHM present in the formulations. Each sample was injected six times after extracting the drug as mentioned above under section 5.5. The results for assay of capsules are given in table-3.

CONCLUSION

We have developed a fast, simple and fully automated analytical method for determination of ZHM in pharmaceutical preparation using HPLC with UV detection. An analytical run takes less than 5.5min. Separation of compounds is very fast, with good reproducibility and peak asymmetry. Validation of this method was accomplished, getting results meeting all requirements. The method is simple, reproducible, with a good accuracy and precision. It allows the analysis of ZHM in bulk, its capsules and in dissolution samples of capsules with a short period of time.

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