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Research Article

DEVELOPMENT AND VALIDATION OF A RAPID RP-HPLC METHOD FOR THE ESTIMATION OF ZIPRASIDONE HYDROCHLORIDE MONOHYDRATE IN DRUG SUBSTANCE AND ITS 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] [1] in pure and its capsule dosage forms. The method was validated as per International Conference on Harmonization [ICH] guidelines[3, 4]. Sunsil C18 column (150×4.6mm, 5µm) was used with a mobile phase containing a mixture of sodium phosphate monohydrate buffer (pH-6.0) and Acetonitrile in the ratio of 40:60% v/v. The analysis was performed with run time of 6 minutes at a flow rate of 2ml/min. The ZHM was monitored at 260nm with UV detection and ZHM was eluted at 2.50min. The method was linear (*r2* =0.999) at concentration ranging from 100 to 300µg/ml, precise (intra-day relative standard deviation [RSD] and inter-day RSD values < 1.0%), accurate (mean recovery = 99.5%), specific and robust. Detection and quantification limits were 0.34 and 1.04µg/ml, estimated from linearity by regression 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.

Keywords: Ziprasidone, RP-HPLC, Validation, Dosage form.

INTRODUCTION

Ziprasidone[5, 7] Hydrochloride Monohydrate [ZHM] is an atypical antipsychotic drug that is chemically unrelated to phenothiazine or butyrophenone antipsychotic agents[5, 7]. 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 (5HT2), dopamine Type 2 (D2), 1 and 2 adrenergic, and H1 histaminergic receptors1. 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].

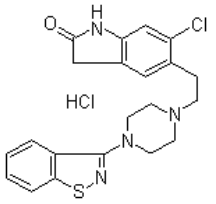

Fig. 1: Chemical Structure of ZHM

A few analytical methods published that describe the quantification of Ziprasidone in plasma by liquid chromatography, fluorescence detection, and UV detection. In the present investigation the authors propose a simple, sensitive and reproducible method for the determination of Ziprasidone hydrochloride monohydrate.

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. This validation study is carried out as per ICH guidelines.

MATERIALS AND METHODS

Instrumentation

Quantitative HPLC was performed on a High performance liquid chromatography equipped with waters 524 binary HPLC pump separation module with manual injector and Waters-2489 dual λ-absorbance UV detector. The data processing was performed using Empower software.

Standards and chemicals

Commercial capsule formulations Zipsydon (Sun Pharma) were used for present study containing 20mg, 40mg and 80mg of ZHM, respectively. HPLC grade Acetonitrile and water as well as monobasic sodium phosphate monohydrate, A.R. grade were purchased from Fisher scientific, 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 Acetonitrile and monobasic sodium phosphate monohydrate buffer (pH-6.0) in the ratio of 60:40% v/v. Stationary phase was Sunsil C18 reverse phase column (150×4.6mm, 5µm) 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 2.0ml/min for 6min. The elute was monitored at 260nm using UV-detector. The retention time of the drug was found to be 2.50min.

Preparation of standard drug solutions

About 21.6mg of Ziprasidone HCl monohydrate was weighed accurately and transferred into a 100mL volumetric flask and dissolved in water and acetonitrile in the ratio of 50:50 v/v(used as diluent). The solution was sonicated for 15min and then the volume made up with a further quantity of the diluent to give 200µg/mL. 20uL of the solution was injected each time into the column for five times the corresponding chromatograms were obtained. From these chromatograms, the retention times and the areas under the peaks of the drug were noted.

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 100mL flask, to this 25mL of methanol, was added and sonicated for 10 min for dispersion of drug from its excipients. Then about 50mL of diluent was added and sonicated for 30minute to get extraction of the drug and finally diluted to 100mL volume to get 200µg/mL. solution. The solutions were filtered through 0.45µ membrane filter before injection and 20µl solution was injected in duplicate injection in to the chromatographic system. From the data amount of ZHM was calculated as monohydrate.

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. 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 blank. Method precision, Recovery in the range of 50% to 150% of label claim of the drug using the blend, Linearity was tested in the range 100–300µg/ml. Intra and inter-day instrumental system.

Precision as well as repeatability and intermediate method precision were obtained using six replicates per day. Limits of detection and quantification were provided for ZHM. Calculation was made by means of RSQ (Residual Square of regression).

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 acetonitrile. Different mobile phases were tried, but satisfactory separation and good symmetrical peak were obtained with the mobile phases consisting of acetonitrile and monobasic sodium phosphate monohydrate buffer (pH-6.0) 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.

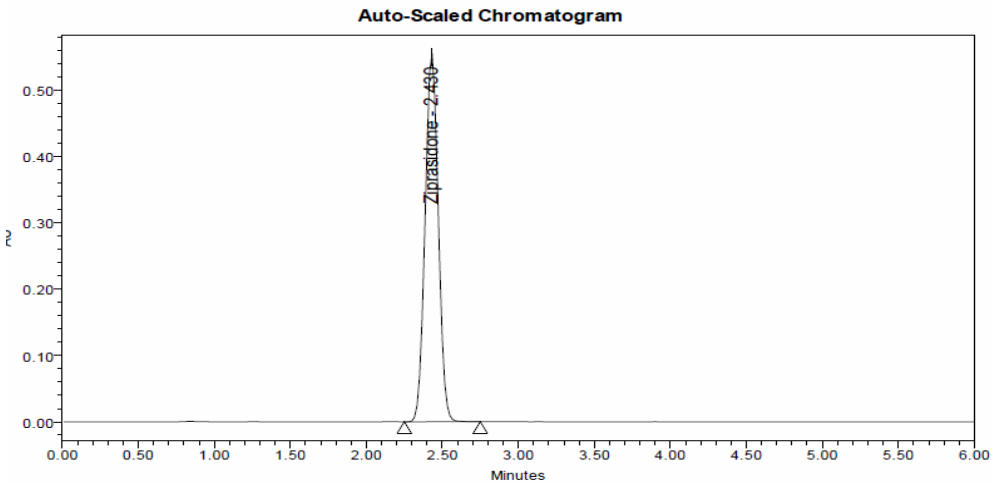

Fig. 2: A typical chromatogram showing the peak of ZHM

Method validation[3, 4]

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

System suitability

For system suitability, five replicates of standard solution were injected and studied the parameters like theoretical plates, tailing factor. The represented data was shown in table-1.

Table 1: System suitability parameters for ZHM by proposed method

Name of the Compound	Theoretical plate	Tailing factor
Ziprasidone	3605	1.04

Specificity

The HPLC chromatograms recorded for the placebo showed almost no peaks at the retention time of ZHM. The peak for ZHM is clearly separated from other excipients of the formulations. As there is no blank interference is not observed at the retention time of ZHM, the HPLC method presented in this study is specific for ZHM.

Precision

In the study of the instrumental system precision where, a RSD of 0.1% was obtained for retention time, and of 0.1% for the area obtained corresponding to the first day, being 0.2% and 0.9% for the second day, respectively. The method precision study for six sample preparations in marked samples showed a RSD of 0.8% and the 95% confidence interval of 1.0.

For the intermediate precision, a study carried out by the same analyst working on different day. The results calculated as inter-day RSD corresponded to 1.0%. The same study was carried out for different analysts (n = 6 number of samples per analyst) obtaining a RSD of 0.9%. 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 in triplicate preparations on composite blend collected from 20 capsules of Zipsidone and analyzed as per the proposed method. The percentage recoveries with found in the range of 98.6 to 100.5 with an overall %RSD of 0.7%. From the data obtained which given in table-2 the method was found to be accurate.

Table 2: Recovery studies for ZHM by proposed method

% Level	Recovery Range	% RSD at each level	Over all %RSD
50	99.1-100.5	0.8	0.7
100	99.3-100.2	0.5	
150	98.6-99.0	0.2	

Linearity

The standard curve was obtained in the concentration range of 100-300µg/ml. The linearity of this method was evaluated by linear regression analysis. Slope, intercept and correlation coefficient [r2] of standard curve were calculated and given in figure-3 to demonstrate the linearity of the method.

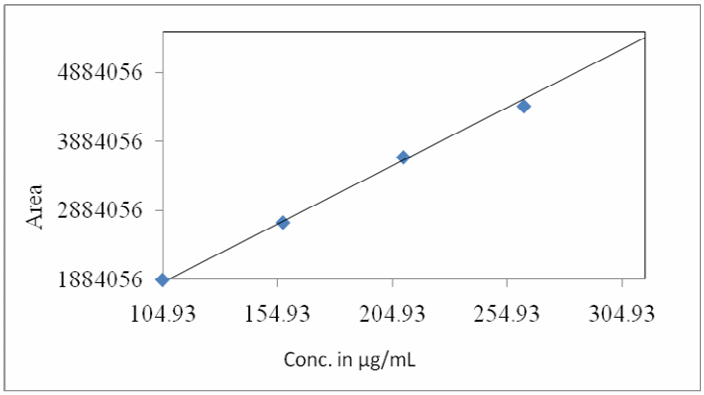

Fig. 3: Calibration curve *for ZHM*

Table 3: Assay results for capsules

Zipsidnone Strength	Avg. Assay for duplicate Sample in mg	Avg. Assay for duplicate Sample in %	% RSD
20mg	20.04	100.3	0.2
40mg	39.95	99.9	0.6
80mg	80.56	100.7	0.3

LOD and LOQ

Limit of detection was found to be 3.44µg/ml and Limit of quantification was found to be 10.42µg/ml .

Assay of the Marketed Samples

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 one manufacturer of three strengths were evaluated for the amount of ZHM present in the formulations. Each sample was analyzed in duplicate preparations 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 reliable analytical method for determination of ZHM in pharmaceutical preparation using HPLC with UV detection. An analytical run takes about 6.0min. 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 with a short period of time.

REFERENCES

1. Raymond Suckow F, Mira Fein, Christoph Corell U and Thomas Cooper B, *Journal of chromatography B*, 2004,**799**, 201-208.
2. Rani BS, Reddy VP, Estimation of Ziprasidone hydrochloride monohydrate in bulk and capsules by reverse phase HPLC, E-Journal of Chemistry, 3, 2006, 169-72.
3. CPMP/ICH/281/95, Q2A, Note for guidance on validation of analytical methods: Definitions and Terminology, CPMP adopted November, 1994.
4. CPMP/ICH/381/95, Q2B, Note for guidance on validation of analytical procedures: Methodology, CPMP adopted December, 1996.
5. Sachse, Julia, Hartter, Sebastian, Hiemke and Christoph, *Therauptic drug monitoring*, 2005, **27**(2), 158-162 .
6. Janiszeski J S, Fouda H G and Cole R O, *Journal of chromatography*, 1995, 668,133.
7. Lasko H A, *Therauptic drug monitoring*, 2001, **23**, 454.
8. Janiszeski J, Schneider R P, Hoffmaster K, Swyden M, Wells D and Fouda H, *Rapid commun.Mass spectrum*.1997, **11**, 1033.