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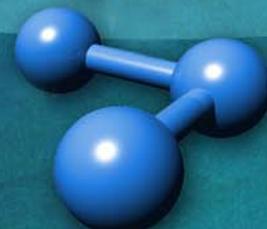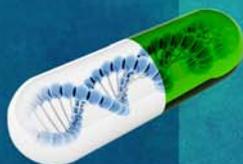
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Research Paper

RP-HPLC METHOD FOR THE ESTIMATION OF ZIPRASIDONE

**Kareti Srinivasa Rao[1]*, Nargesh Kumar Keshar[1],
Prasenjit Roy Choudhury[1], M E Bhanoji Rao[1] and Ajay Kumar Pattnaik[2]**

*Corresponding Author: **K. Srinivasa Rao,** ✉ *ksrao108@gmail.com*

A simple, sensitive, accurate and precise LC assay method was developed for the quantitative determination of Ziprasidone Hydrochloride (ZSH) in pharmaceutical dosage form. Chromatographic separation was achieved by use of Lichrospher RP-18 column (250 × 4.0 mm, 5 m), using 20 mM ammonium acetate (pH adjusted to 3.0 with orthophosphoric acid) and methanol (30:70 %v/v) as mobile phase at 25°C. The described method was linear over a range of 1-500 g mL-1 for determination of ZSH (r= 0.9998). F-test and t-test at 95% confidence level were used to check the intermediate precision data obtained under different experimental setups; the calculated value was found to be less than critical value. The developed method was found to be simple, specific, robust, linear, precise, and accurate for the determination of ZSH in pharmaceutical formulations.

Keywords: Ziprasidone Hydrochloride, validation, assay, recovery studies.

INTRODUCTION

Ziprasidone, 5-[2-[4-(1, 2-benzisothiazol-3.2-yl)-1-piperazinyl] ethyl]-6- chloro-1,3.2-dihydro-2 *H* -indol-2-one (ZSH) (Figure 1) is an antipsychotic agent that is chemically unrelated to phenothiazine or butyrophenone antipsychotic agents. ZSH 's unique pharmacology offers advantages in the areas of antipsychotic-induced weight gain and possibly the treatment of depressive symptoms in patients with schizophrenia or schizoaffective disorder. The efficacy of ZSH in schizophrenia is primarily attributable to dopamine (i.e, D_2) and serotonin (specifically $5HT_2A$) receptor antagonism, which contributes to its more favorable effect on weight gain than clozapine, olanzapine, and risperidone (Sweetman, 2009).

Literature survey revealed that several analytical methods were reported for the determination of ZSH in biological matrices, including; liquid chromatography-tandem mass spectrometry (Zhang *et al.*, 2008; Zhang *et al.*, 2007; Zhang *et al.*, 2007; Al-Dirbashi *et al.*, 2006), liquid chromatography/UV detection (Zhang *et al.*, 2007), liquid chromatography/fluorescence detection (Suckow *et al.*, 2004). Few analytical

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[1]   Roland Institute of Pharmaceutical Sciences, Berhampur -760010, Orissa, India.

[2]   Khallikote College, Berhampur, Orissa, India.

Figure 1: Chemical Structure of Ziprasidone
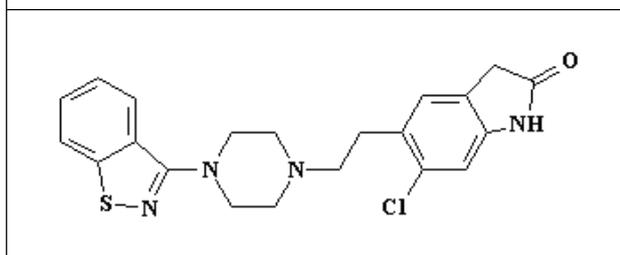
methods have been reported for the determination of ZSH in raw material and pharmaceutical formulations including; TLC densitometry (El-Sherif *et al.*, 2004), HPLC/UV detection(El-Sherif *et al.*, 2004; Rani and Reddy, 2006; Prasanthi and Rao, 2010), capillary zone electrophoresis (Cecilia *et al.*, 2008) and spectrophotometric methods (Srinubabu *et al.*, 2006; Anand kumar *et al.*, 2010; Kishore and Hanumantharao, 2010; Chudasama *et al.*, 2011).

Although the reported HPLC methods for estimation of ZSH in pharmaceutical formulations present adequate linearity, precision, and recovery, they show a series of limitations including lack of sensitivity, which results in the lower limit of quantification and long chromatographic times. However, these methods are relatively non-specific, laborious, time consuming and have long retention times. However, the present study achieved satisfactory results in terms of selectivity, linearity, precision and accuracy under simple chromatographic conditions. The present work describes the validation parameters stated by the International Conference on Harmonization [ICH] guidelines [(ICH, 1994; 1996) includes specificity, linearity, range, accuracy, precision, robustness to achieve analytical methods with acceptable characteristics of suitability, reliability and feasibility.

MATERIALS AND METHODS

Materials

ZSH reference standard was obtained from Torrent pharma, India. ZSH commercial tablets (Zipsydon 40 mg) were purchased from the local market. HPLC grade methanol was purchased from Rankem, India, and high pure water was prepared by using Millipore Milli Q plus purification system. Ammonium acetate and orthophosphoric acid, both of A.R. grade, were purchased from Merck Ltd. (Mumbai, India).

Apparatus and Chromatographic Conditions

Quantitative HPLC was performed on Shimadzu HPLC with LC 10 AT VP series pumps besides SPD 10 A VP UV-Visible detector. The output signal was monitored and integrated using Shimadzu CLASS-VP Version 6.12 SP1software. The chromatographic separations were performed using Lichrospher RP-18 column (250 mm × 4mm × 5 µm) maintained at ambient temperature, eluted with mobile phase at a flow rate of 1 mL/min for 15 min. The mobile phase consisted of 20 mM ammonium acetate (pH adjusted to 3.0 with orthophosphoric acid) and methanol (30:70 %v/v). Measurements were made with injection volume 20µl and ultraviolet (UV) detection at 225 nm.

Preparation of standard and sample solutions

Stock solution of ZSH (1mg/mL) was prepared by dissolving 25 mg of ZSH in 25 mL of volumetric flask containing 10 mL of methanol. The solution was sonicated for about 30 min and then made up to volume with mobile phase. Working standard solutions of ZSH were prepared by taking suitable aliquots of ZSH stock solution and diluted to 10 mL with mobile phase in a 10 mL volumetric

flask to yield the drug concentrations in the range of 1-500 mg mL^{-1}.

To prepare a sample solution, twenty weighed tablets of zypsydon (40 mg of ZSH) were ground and an amount of powder equivalent to 10 mg of active compound was diluted with methanol and then sonicated for 20 min. The sample solution was filtered and the appropriate aliquot was diluted in the mobile phase to obtain a final solution containing 10 mg mL^{-1} of ZSH.

METHOD VALIDATION

The validation procedure for the analysis of ZSH by LC method followed the International Conference on Harmonization (ICH) guideline and United States Pharmacopoeia. The performance parameters evaluated in this method were specificity, robustness, linearity, limit of detection (LOD), limit of quantitation (LOQ), precision, and accuracy.

Robustness

Chromatographic parameters (peak retention time, theoretical plates, tailing factor, retention factor, and repeatability) were evaluated using both samples and reference substance solutions (10 mg mL^{-1}) changing wavelength (221 and 229 nm), column temperature (23 and 27 °C), flow rate (0.8 and 1.2 mL min^{-1}) and methanol concentration (65 and 75%).

Linearity

Linearity was established by least squares linear regression analysis of the calibration curve. The constructed calibration curves (n=3) were linear over the concentration range of 1-500 μg/mL. Peak areas of ZSH was plotted against their respective concentrations and linear regression analysis was performed on the resultant curve.

LOD and LOQ

LOD and LOQ were determined by reducing the concentration of a standard solution until the ZSH peak response was approximately three or ten times, greater than the noise, respectively.

Precision

The precision of the proposed method was evaluated by carrying out six independent (50μg/mL) assays of test sample. RSD (%) of six assay values obtained was calculated. Intermediate precision was carried out by analyzing the samples by a different analyst on another instrument.

Accuracy

The accuracy of the method was determined through the recovery test of the samples, using known amounts of ZSH reference standard. For LC method, aliquots of 0.8, 1.0 and 1.2 mL of a ZSH standard solution (100 mg mL^{-1}) were added to three sample solutions containing a fixed amount of ZSH (100 mg) in mobile phase, respectively. Therefore, this recovery study was performed at a final concentration solution of 80%, 100% and 120% level of ZSH. All solutions were prepared in triplicate and analyzed.

System suitability test

System suitability tests were performed to ensure that the LC system and procedure are capable of providing quality data based on USP 31 requirements. The system suitability parameters include ZSH retention time, tailing factor and number of theoretical plates, as well as the peak area relative-standard deviation (RSD, n= 6) of reference standard.

RESULTS AND DISCUSSION

Optimization of LC method

To develop a suitable and robust HPLC method for the determination of ZSH in different mobile phases - Phosphate buffer (pH 5.0) :Methanol, Phosphate buffer (pH 3.0) : Acetonitrile, Ammonium Acetate buffer (pH 5.0) : Acetonitrile, Phosphate buffer (pH 3.0) : Acetonitrile, Ammonium Acetate buffer (pH 3.0) : methanol with different compositions at different flow rates (0.5, 0.75, 0.8, 1.0, 1.2, 1.5, mL/min) and different detection wavelength were tried. The mobile phase using 20 mM ammonium acetate (pH adjusted to 3.0 with orthophosphoric acid) and methanol (30:70 %v/v) at a flow rate of 1.0 mL/

min gave peaks with good resolution for ZSH are eluted at retention time around 4.76 min and with symmetric peak shape as shown in Figure 2.

Method Validation

Robustness

The robustness of the method was examined by small variations of critical parameters, and percent of ZSH, retention time (R_t), number of theoretical plates (*N*) and tailing factor (*T*), were evaluated (Table 1).

The robustness study has been proved that in every employed condition, the chromatographic parameters agreed with established values and the assay data remained acceptable. A tailing

Figure 2: Representative chromatogram of ZSH in Pharmaceutical Formulation
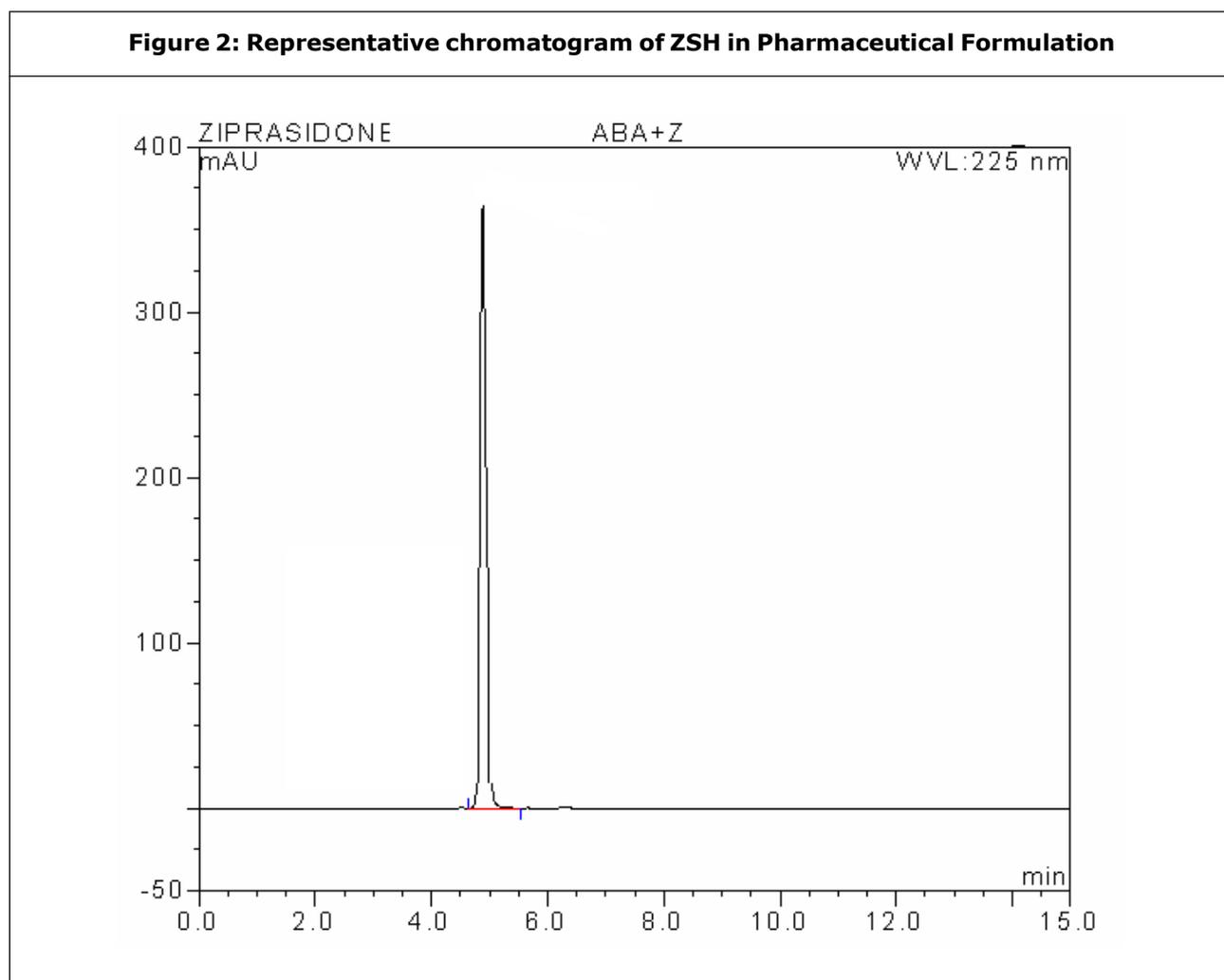
Table 1: Robustness Experiments of LC Method for Determination of ZSH

Chromatographic Parameter	Condition	ZSH(%)	R_t^a ZSH(min)	N^b	T^c
Wavelength (nm)	220	98.19	7.48	2901	1.22
	230	98.73	7.32	3015	1.17
Temperature (°C)	23	99.09	7.14	3420	1.05
	27	98.47	7.22	3309	1.19
Flow rate (mL min⁻¹)	0.8	99.18	7.350	3207	1.21
	1.2	101.05	7.099	3197	1.33
methanol (%)	68	100.59	7.163	3213	1.04
	72	100.17	7.477	3105	1.21
	Normal^d	100.09	7.461	3288	1.12

Note: ^{a} R_t: retention time, ^{b} N: number of theoretical plates, ^{c} T: tailing factor, ^{d} Normal condition (mobile phase): Lichrospher RP-18 column (250 mm × 4mm × 5 μm), 20 mM ammonium acetate (pH adjusted to 3.0 with orthophosphoric acid) and methanol (30:70 %v/v), flow rate 1.0 mL min⁻¹; UV detection at 225 nm.

factor of 1.12 refers to a symmetric peak. The calculated values for the tailing factor for each chromatographic condition were in the acceptable range of $0.8 \leq T \leq 1.5$. The number of theoretical plates demonstrated the measure the column efficiency in different conditions. Flow rate (0.8 and 1.2 mL min⁻¹) and percent of methanol (68 and 72%) resulted in changes in the retention time in comparison with the proposed normal condition. However, no significant changes were observed regarding quantification of ZSH.

Linearity

The standard curves for ZSH were constructed and demonstrated to be linear in the concentration range of 1-500 mg mL⁻¹. The representative linear equation Y= 34616+7412.9, where x is the concentration (mg mL⁻¹) and y is the peak area. The correlation coefficient was r= 0.9998. Linearity data were validated by the analysis of variance (ANOVA), which demonstrated significant linear regression and no significant linearity deviation ($p < 0.05$).

LOD and LOQ

The limit of quantitation (LOQ) of the present method was found to be 1.0 µg/mL with a resultant %RSD of 0.74% (n = 5). The limit of detection (LOD) was found to be 0.46 µg/mL. This low values obtained were indicative of the high sensitivity of the method.

Precision

Precision values obtained for the determination of ZSH in samples with their RSD were shown in Table 2. F-test and t-test was applied to the two

Table 2: Precision of ZSH by Proposed Method

Precision	ZSH
	Mean assay (%)/%R.S.D
Set 1(n=6)	98.8/0.829
Set 2(n=6)	99.6/1.191
	Calculated value/ critical value
F-test	1.931/3.368
t-test	1.718/2.106

Table 3: Recovery of Standard Solution Added to Commercially Available Sample

Amount Added (μg mL^{-1})	Amount Found (μg mL^{-1})	% Recovery[a] $\pm$ RSD	Mean % Recovery
80.0	79.58	99.48 $\pm$ 0.22	
100.0	99.10	99.10 $\pm$ 0.21	99.52
120.0	119.97	99.98 $\pm$ 0.18	

Note: [a] Each value is a mean of three determinations.

sets of data at 95% confidence level, and no statistically significant difference was observed.

Accuracy

Accuracy was evaluated by the simultaneous determination of the analyte in solutions prepared by the standard addition method. Three different concentrations of ZSH standard were added to Zypsydon solution. The mean recovery was shown (Table 3) and this value showed that the method was accurate.

System suitability test

The system suitability parameters evaluated, under the experimental conditions, showed a single peak of the drug around 4.7 min, tailing factor (T = 1.12) and number of theoretical plates (N = 4217), as well as the peak area relative-standard deviation (RSD= 1.04%, n = 6).

Assay

The validated method was applied to the determination of ZSH in commercially available Zypsydon 40 mg tablets. The results of the assay (n = 9) undertaken yielded 99.32% (%RSD = 0.93%) of label claim for ZSH. The observed concentration of ZSH was found to be 39.73±14.8 μg/mL (mean±SD). The mean retention time of ZSH was 4.7 min. The results of the assay indicate that the method is selective for the analysis of ZSH without interference from the excipients used to formulate and produce these tablets.

CONCLUSION

The proposed method was found to be simple, precise, accurate and rapid for determination of ZSH from pure and its dosage forms. The mobile phase is simple to prepare and economical. The sample recoveries in all formulations were in good agreement with their respective label claims and they suggested non-interference of formulation excipients in the estimation. Hence, this method can be easily and conveniently adopted for routine analysis of ZSH in pure form and its dosage forms and can also be used for dissolution or similar studies.

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