Scholars Academic Journal of Pharmacy (SAJP) **ISSN 2320-4206**
Sch. Acad. J. Pharm., 2013; 2(5):360-364

(An International Publisher for Academic and Scientific Resources)
www.saspublisher.com

Research Article

Development and Validation of New RP-HPLC Method for the Determination of Zolpidem Tartrate in Pure and Pharmaceutical Formulations

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Abstract: The present work is concerned with application of simple, precise, accurate, reproducible and specific RP-HPLC method for estimation of Zolpidem Tartrate (ZPT) in bulk and pharmaceutical formulations. Separation of ZPT was successfully achieved on a Symmetry XTerra C18 (4.6 x 150mm, 5 µm) Waters or equivalent in an isocratic mode utilizing Ammonium Acetate Buffer (4.5 pH): Methanol (40:60%v/v) at a flow rate of 0.8 mL/min and eluate was monitored at 300 nm, with a retention time of 3.14 minutes. The method was validated and the response was found to be linear in the drug concentration range of 20 µg/mL to 60 µg/mL. The values of the slope, intercept and the correlation coefficient were found to be 47336, 13206 and 0.999 respectively. The RSD values for system precision and method precision were found to be 0.14 % (Intra-day), 0.18% (Inter-day) and 0.46 % (Intra-day), 0.14 % (Inter-day) respectively. Accuracy of method was determined through recovery studies which were found to be 98.90-100.87%.
Keywords: Zolpidem Tartrate(ZPT), RP-HPLC, Acetate Buffer (4.5 pH), Methanol.

INTRODUCTION

Zolpidem (Tartrate) is a non-benzodiazepine sedative-hypnotic for the short-term treatment of insomnia. Although chemically unrelated to other hypnotics such as the benzodiazepines or barbiturates, zolpidem does share some pharmacological actions with these drugs [1,3]. Unlike the benzodiazepines, zolpidem produces muscle relaxation and anticonvulsant effects only at doses much higher than the hypnotic dose. Zolpidem[2] has a short half-life and no active metabolites. Chemically, It is N,N,6- trimethyl-2-p-tolylimidazo[1,2-a] pyridine-3-acetamide L-(+)-tartrat (Fig. No.1) It is a white to off-white crystalline powder that is sparingly soluble in water, alcohol, and propylene glycol. It produces agonistic effect on $GABA_A$ receptors and it is used in the treatment of insomnia. Zolpidem (ZPT) belongs to a class of medications called sedative-hypnotics [[4].

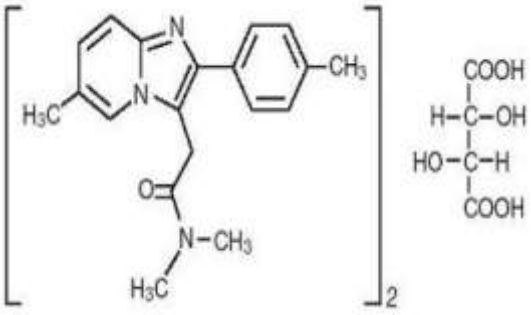

Figure.No.1: Chemical structure of zolpidem(Tartrate)

Literature survey reveals that a few HPLC methods, Potentiometric method , UV spectrophotometric, LC-MS, method have been used [5-7].The objective of the present work was to develop simple, rapid, accurate, specific and economic RP-HPLC stability indicating method.

The aim of the present work was to develop and validate a simple, fast and reliable isocratic RP HPL C18 method with UV detection for the determination of Zoplidem in bulk and in tablet dosage forms. The important features and novelty of the proposed method included simple sample treatment with sonication of small amount of powder sample at ambient temperature, short elution time (less than 5 min) ZPT, good precision (R.S.D.less than 2%) and high recovery (greater than 98%). Confirmation of the applicability of the developed method validated according to the International Conference on Harmonization (ICH) [8] for the determination of ZPT in bulk and in tablet dosage form.

MATERIALS AND METHODS

Chemicals

Zolpidem Tartrate (ZPT) was obtained from Abbott Pharmaceuticals. (India) and was used as such without further purification. The commercial formulations available are Zolfresh (5 mg and 10 mg) and were purchased from the local market.

Reagents

Ammonium Acetate (AR) (Thomas baker), Glacial Acetic Acid (AR) (Merck), Methanol (HPLC) (Merck), Acetonitrile (HPLC) (Merck), Water (HPLC) (Loba Chemi).

Instruments and Equipments

WATERS HPLC, Model: Aliance 2695, UV-Visible Dual absorbance Detector 2487, with a Automated Sample injector. The output signal was monitored and integrated using Empower 2 software. A Symmetry XTerra C18 (4.6 x 150mm, 5 µm, Make: Waters), UV-3000$^+$ LABINDIA Double beam with UV win 5 software UV-Visible spectrophotometer with 1cm matched quartz cells, Weighing Balance, Sonicator, pH Meter, Heating Mantle, Filter Paper 0.45 microns.

Preparation of buffer

Weigh 7.0 grams of Ammonium Acetate in to a 1000mL beaker, dissolve and diluted to 1000mL with HPLC water. The solution adjusted the pH 4.5 with Glacial Acetic acid.

Preparation of mobile phase

Mix a mixture of above buffer 400mL (40%) and 600 mL of Methanol HPLC (60%) and degas in ultrasonic water bath for 5 minutes. Filter through 0.45 µ filter under vacuum filtration. The typical chromatogram of mobile phase is as show in Fig 6.5 respectively.

Preparation of standard solution

Stock solution of ZPT (1 mg/mL) was prepared by weighing 10 mg and dissolving in the mobile phase Ammonium Acetate Buffer (4.5pH): Methanol (40:60%v/v). Standards solutions of ZPT were prepared in the range of 20 µg/mL to 60 µg/mL by diluting the stock solution with mobile phase. The eluate was monitored at 300nm. Each solution was then injected into the column and the chromatograms were recorded.

Preparation of sample solution

Twenty tablets were weighed to get the average weight and then powdered. The fine powder, equivalent to 10 mg of ZPT, was weighed and transferred into a 10 mL calibrated volumetric flask and dissolved using mobile phase. This mixture was sonicated (30 min) and then filtered through a 0.45 µm Whatman filter paper. After filtration, Aliquots solutions were prepared by taking 1mL into 10mL volumetric flasks, separately and made up to volume with mobile phase to yield concentrations of drug in range of linearity previously described. The amount of ZPT was calculated from the related linear regression equations.

RESULTS AND DISCUSSION

Method Development

The method utilizing Methanol: Ammonium acetate buffer of pH 4.5as mobile phase in different ratios yielded sharp peak, whereas with MeOH: Acetate buffer in 40:60% v/v dilutions symmetric peak was obtained at flow rate 0.8mL/min and wavelength is 300nm. The peak was shown in figure no.2 and optimized column parameter shown in the table no.1

Validation

Validation of HPLC method was in compliance with recommendations of the ICH Guidelines.

Linearity

For all methods, 5-point calibration curve were prepared on single day. The results obtained were used to calculate the equation of the line by using linear regression by the least square method. The results of these are shown in table no.2.

Procedure for calibration curve

Prior to injection of the drug solutions, the column was equilibrated with the mobile phase flowing through the systems. The chromatographic separation was achieved using a mobile phase consisting of Ammonium Acetate Buffer: Methanol (40:60 v/v) at a flow rate of 0.8mL/min. The eluent was monitored using UV detection at a wavelength of 300 nm. The column was maintained an ambient temperature (25°C) and an injection volume of 20µl of each of standard and sample solutions were injected into the HPLC system to get the chromatograms. The retention time and peak areas of the drug were recorded. The calibration curve for the HPLC analysis was constructed by plotting the peak area of normalization of ZPT on x-axis against concentration on y-axis**.** The obtained graph was shown in fig.3

Precision

Intra-day precision of the method was determined by repeat analysis (three identical injections) at three concentration levels. Inter-day precision was established by performing the analysis next day on freshly prepared solution. The low RSD values of table 222 indicate the ruggedness of the method. The low RSD values indicate the ruggedness of the method. [Table no.3 to 6].

Accuracy

The accuracy of an analytical method is the closeness of the test results to the true value. It has been determined by application of the analytical procedure to recovery studies, where known amount of standard ZPT (50%, 100%, and 150%) is spiked into the pre-analyzed amount of formulation of concentration 40µg/mL. From this percentage recovery values were calculated [Table-7].

Robustness

Robustness of the method reflects the reliability of an analysis with respect to deliberate variations in the method parameters. Here, the flow rate and mobile phase composition were slightly changed to lower and higher sides of the actual values to find if the change in the peak area and retention time were within limits. The results obtained with changes in the parameters on a 40µg/mL solution are as shown in Table No. 8 respectively.

System suitability parameters

System suitability parameters can be defined as tests to ensure that the method can generate results of acceptable accuracy and precision. The requirements for system suitability are usually developed after method development and validation has been completed. The USP (2000) defines parameters that can be used to determine system suitability prior to analysis. The system suitability parameters like Theoretical plates (N), Asymmetry (A), LOD(µg/mL) and LOQ(µg/mL) were calculated and compared with the standard values to ascertain whether the proposed RP-HPLC method for the estimation of ZPT in pharmaceutical formulations was validated or not. [Table-9].

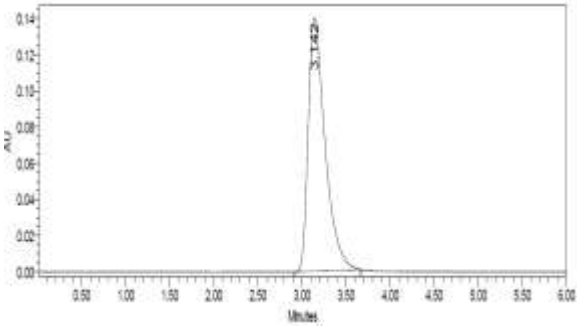

Fig. No.2: A model chromatogram for Zolpidem

Table No.1: Optamized Method Parameters

Parameters	Method
Column(Stationary Phase)	Symmetry C18 (4.6 x 150mm, 5 µm, Make: XTerra) or equivalent
Mobile Phase	Ammonium Acetate Buffer (4.5 pH):Methanol
Flow rate (mL/min)	0.8
Run time (min)	6
Column temperature(°C)	Ambient
Volume of injection loop (µl)	20
Detection wavelength (nm)	300
Drug RT (min)	3.14
Linearity range (µg/mL)	20-60
Regression equation	
Slope	47336
Intercept	13206
Correlation coefficient	0.999

Table No.2: Linearity range of proposed RP-HPLC method

S.No	Linearity Level	Concentration	Area
1	I	20µg/mL	926213
2	II	30µg/mL	1402091
3	III	40µg/mL	1862724
4	IV	50µg/mL	2352834
5	V	60µg/mL	2844035
Correlation Coefficient			0.999

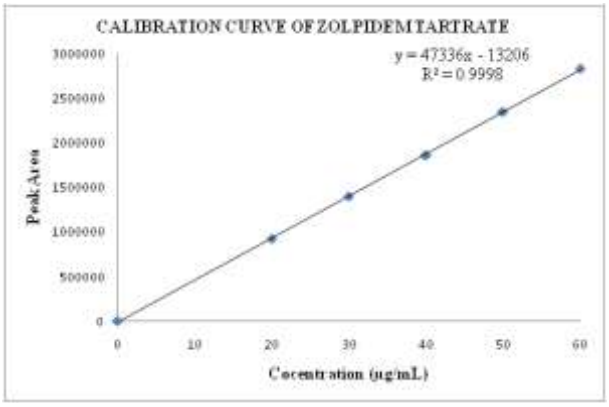

Fig.No.3: Linearity plot of ZPT

Table No. 3: System Precision (Intra-day)

Injection	Area
Injection-1	1892556
Injection-2	1898440
Injection-3	1896326
Injection-4	1896644
Injection-5	1899562
Average	1896705
Standard Deviation	2670.8
%RSD	0.14

Table No. 4: System Precision (Inter-day)

Injection	Area
Injection-1	1858787
Injection-2	1851176
Injection-3	1851848
Injection-4	1851874
Injection-5	1851123
Average	1852962
Standard Deviation	3275.6
%RSD	0.18

Table No. 5: Method Precision (Intra-day)

Injection	Area
Injection-1	1910172
Injection-2	1912204
Injection-3	1918943
Injection-4	1920794
Injection-5	1928479
Injection-6	1932712
Average	1920551
Standard Deviation	8840.8
%RSD	0.46

Table No. 6: Method Precision (Inter-day)

Injection	Area
Injection-1	1882144
Injection-2	1881694
Injection-3	1880161
Injection-4	1882126
Injection-5	1886945
Injection-6	1879498
Average	1882095
Standard Deviation	2614.2
%RSD	0.14

Table No. 7: Accuracy

Sample ID	Concentration (µg/mL)		%Recovery	Statistical Analysis
	Pure drug	Formulation		
S_1 : 50 %	20	40	100.84	**Mean** = 100.87 **SD** = 0.1868 **%RSD** = 0.185
S_2 : 50 %	20	40	100.70	
S_3 : 50 %	20	40	101.07	
S_4 : 100 %	40	40	98.47	**Mean** = 99.65 **SD** = 0.3175 **RSD** = 0.318
S_5 : 100 %	40	40	99.02	
S_6 : 100 %	40	40	98.47	
S_7 : 150 %	60	40	99.09	**Mean** = 98.90 **SD** = 0.2753 **%RSD** = 0.278
S_8 : 150 %	60	40	98.59	
S_9 : 150 %	60	40	99.04	

Table No. 8: Robustness

Sl. No.	Parameter	Condition	Peak area		Statistical analysis	Retention time		Statistical analysis
1	**Flow rate (mL/min)**	0.7	1943638	Mean= **1942204**	**Mean=** 1921616 **SD=** 18550 **%RSD=** 0.965	3.159	Mean= **3.156**	**Mean=** 3.128 **SD=** 0.032 **%RSD=**1.023
			1942987			3.153		
			1939987			3.156		
		0.8	**1916444**			**3.137**		
		0.9	1905145	Mean= **1906202**		3.096	Mean= **3.092**	
			1904868			3.096		
			1908594			3.090		
2	**Mobile phase ratio**	45:55	1976731	Mean= **1975874**	**Mean=** 1950589 **SD=** 30689 **%RSD=** 1.573	3.159	Mean= **3.159**	**Mean=** 3.130 **SD=** 0.031 **%RSD=**0.990
			1975050			3.161		
			1975843			3.158		
		40:60	**1916444**			**3.137**		
		35:65	1958561	Mean= **1959450**		3.095	Mean= **3.096**	
			1958974			3.098		
			1960871			3.096		

Table No. 9: System Suitability Parameters

Parameters	**Obtained values**
Theoretical plates (N)	2405.28
Asymmetry	1.65
LOD (µg/mL)	0.036
LOQ (µg/mL)	0.12

CONCLUSION:

The proposed method was found to be simple, precise, accurate, rapid and specific for determination of Zolpidem Tartrate from pure and its dosage forms. The mobile phase is simple to prepare and economical. The sample recoveries in the formulation 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 Zolpidem Tartrate in pure form and its dosage form and also can be used for dissolution or similar studies.

ACKNOWLEDGEMENT

Authors greatfully acknowledge the founder Chairman Mr. M. Mohan Rao and Principal Dr. N. Srinath Nissakara Rao of Sri Siddhartha Pharmacy College, Nuzvid for their kind help and providing all necessary facilities and also thank Richer Laboratories, Hyderabad for providing the gift sample of Zolpidem.

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