

STABILITY INDICATING RP-HPLC METHOD FOR THE ESTIMATION OF ZOLPIDEM TARTRATE IN BULK AND TABLET DOSAGE FORM

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ABSTRACT

A novel, simple and economic reverse phase high performance liquid chromatography (RP-HPLC) Stability indicating method has been developed for the estimation of zolpidem tartrate in bulk and tablet dosage form with greater precision and accuracy. Separation was achieved on C18 column (250X4.6mm i.d.,5 μ m) in isocratic mode using methanol and acetonitrile in the ratio 50:50 (v/v) as mobile phase, pumped in to the column at flow rate of 1.0 mL min⁻¹ and the detection of eluent from the column was carried out using variable wavelength UV detector at 293 nm. The total run time was 10 min and the column was maintained at ambient temperature. The retention time of zolpidem tartrate was 3.467 min. The standard curves were linear over the concentration range of 10-60 μ g mL⁻¹ with $r^2= 0.999$ and the LOD and LOQ values for zolpidem tartrate were 0.026 μ g mL⁻¹ and 0.056 μ g mL⁻¹, respectively. The percentage recovery was found to be 99.06 to 100.60 and the % RSD of intraday and inter day precision was found to be 0.68 and 0.61, respectively. The percentage amount of a marketed tablet formulation of zolpidem tartrate was found to be 99.08 %. Forced degradations were carried out under thermolytic, photolytic, acid and base hydrolytic and oxidative stress conditions. In the stressed sample chromatograms, it demonstrated the specificity of the assay method for their estimation in presence of degradation products. The method was validated as per ICH guidelines. Validation studies demonstrated that the proposed RP-HPLC method is simple, specific, rapid, reliable and reproducible. The high recovery and low relative standard deviation confirm the suitability of the proposed method for the routine quality control analysis of zolpidem tartrate in bulk and tablet dosage forms and is stability indicating.

Keywords: Zolpidem tartrate (ZOL), RP-HPLC, Method Development, Validation, Stability Indicating, ICH guidelines.

INTRODUCTION

Zolpidem tartrate (ZOL) is a non-benzodiazepine hypnotic of the imidazopyridine class and is available in 5 mg and 10 mg strength tablets for oral administration. Chemically, it is N,N,6-trimethyl-2-p-tolylimidazo[1,2-a]pyridine-3-acetamide L-(+)-tartrate. (Fig. 1) The drug is official in British Pharmacopoeia¹ and Merck Index². 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 belongs to a class of medications called sedative-hypnotics³. Literature survey reveals that four HPLC methods⁴⁻⁷, one potentiometric method⁸ one spectrophotometric method⁹ have been developed for the estimation of zolpidem tartrate in human serum and tablet formulation. Stability testing plays an important role in the process of drug development. The purpose of stability testing is to provide confirmation on how quality of a drug substance varies with time under the influence of a variety of environmental factors such as temperature, humidity, and light and enables recommendation of storage conditions, and shelf life to be established. The assay of drug product in stability test sample needs to be determined using stability indicating method, as recommended by the international Conference on Harmonization (ICH).

The objective of the present work was to develop simple, rapid, accurate, specific and economic RP-HPLC stability indicating method¹⁰ for the estimation of Zolpidem tartrate in bulk and tablet. The method was further validated as per ICH guidelines¹¹ for the parameters like precision, accuracy, sensitivity, and linearity. The results of analysis were validated statistically and by recovery studies. These methods of estimation of Zolpidem tartrate were found to be simple, precise, accurate and economic.

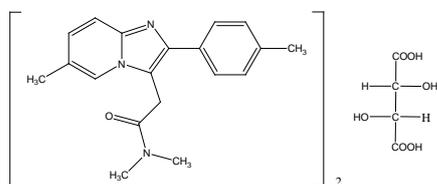


Fig. 1: Structure of Zolpidem Tartrate

MATERIALS AND METHODS

Samples

Zolpidem tartrate, N,N,6-trimethyl-2-p-tolylimidazo[1,2-a]pyridine-3-acetamide L-(+)-tartrate, was kindly provided by Lupin Pharmaceuticals Ltd. Jammu and Kashmir, India. The pharmaceutical formulation used in this study was procured from local market.

Reagents

Methanol and acetonitrile were of HPLC grade, from Thomas Baker (India). sodium hydroxide (NaOH), hydrochloric acid (HCl), and hydrogen peroxide (H₂O₂) were from Qualigens Fine Chemicals (Glaxo Ltd. Mumbai, India). Water was bidistilled. All other chemicals used were analytical or HPLC grade.

Apparatus

A Jasco HPLC system (Japan) composed of an PU-2080 plus pump equipped with a 7725i Rheodyne (CA, USA) injector, an UV-2075 plus UV-vis detector and a LC-Net II/ADC with inbuilt Borwin software.

Chromatographic conditions

The separation was performed on a 25 cm×4.6mm i.d. HiQ Sil-C18 HS column (Kya Tech, Japan). The flow rate was 1.0 mL min⁻¹. The injection volume was 20 μ l. The detection wavelength was set at 293 nm. The mobile phase consisted of acetonitrile and methanol (50:50, v/v). The run time was set at 10 min and column temperature was maintained at ambient. Prior to injection of analyte, the column was equilibrated for 30 min with mobile phase. The mobile phase was premixed, filtered through 0.45 μ m membrane filter and degassed by sonication.

Method Validation

Linearity

A stock solution of ZOL (1000 μ g mL⁻¹) was prepared by dissolving 100 mg drug in 100 ml mobile phase then solutions of different concentrations (10–60 μ g mL⁻¹) for construction of calibration plots were prepared from this stock solution. The mobile phase was filtered

through a 0.45 μm membrane filter and delivered at 1.0 mL min^{-1} for column equilibration; the baseline was monitored continuously during this process. The detection wavelength was 293 nm. The prepared dilutions were injected in series, peak area was calculated for each dilution, and concentration was plotted against peak area.

Accuracy

Accuracy was determined by the standard addition method. Previously analyzed samples of ZOL ($10 \mu\text{g mL}^{-1}$) were spiked with 80, 100, and 120% extra ZOL standard and the mixtures were analyzed by the proposed method. The experiment was performed in triplicate. Recovery (%), RSD (%), and standard error (SE) were calculated for each concentration.

Precision

Precision was determined as both repeatability and intermediate precision, in accordance with ICH recommendations. Repeatability of sample injection was determined as intra-day variation and intermediate precision was determined by measurement of inter-day variation. For both intra-day and inter-day variation, solutions of ZOL at single concentrations ($10 \mu\text{g mL}^{-1}$) was determined.

Reproducibility

The reproducibility of the method was checked by determining precision on a different column, analysis being performed by another analyst. For both intra-day and inter-day variation, solutions of ZOL at single concentrations ($10 \mu\text{g mL}^{-1}$) were determined six times.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD and LOQ were determined by the standard deviation ($S_{y/x}$) method. LOD and LOQ were determined from the slope, S , of the calibration plot, $S_{y/x}$, by use of the formulae $\text{LOD} = 3.3 \times S_{y/x}/S$ and $\text{LOQ} = 10 \times S_{y/x}/S$.

Robustness

The robustness of the method was determined to assess the effect of small but deliberate variation of the chromatographic conditions on the determination of ZOL. Robustness was determined by changing the mobile phase flow rate to 0.9 and 1.1 mL min^{-1} and the concentration of methanol in the mobile phase to 48 and 52%.

Procedure for Forced Degradation Study of Drug Substances

Forced degradation were carried out under thermolytic, photolytic, acid and base hydrolytic, and oxidative stress conditions.

Acid degradation

About 10 mg of ZOL was accurately weighed and dissolved in 10 mL of mobile phase, then 10 mL of 1 N HCl was added. The mixture was kept at room temperature for 48 h, and then it was neutralized with 1 N NaOH to pH 7. The volume was made up to 50 mL with mobile phase.

Alkali Degradation

About 10 mg of ZOL was accurately weighed and dissolved in 10 mL of mobile phase, then 10 mL of 1 N NaOH was added, and the mixture was kept at room temperature for 48 h. It was neutralized with 1 N HCl to pH 7, and the volume was made up to 50 mL with mobile phase.

Oxidative degradation

About 10 mg of ZOL was accurately weighed and dissolved in 10 mL of mobile phase. Then 10 mL of 3 % H_2O_2 was added and the

mixture kept at room temperature for 48 h. Further the volume was made up to 50 mL with mobile phase.

Thermal degradation

About 50 mg of drug substance kept at 80°C for 48 h. Then the solution was prepared to achieve $100 \mu\text{g mL}^{-1}$ of ZOL.

UV degradations

About 50 mg of drug substance was exposed to UV short (254 nm) and UV long (366 nm) light for 48 h. Then, the solution was prepared to achieve $100 \mu\text{g mL}^{-1}$ of ZOL.

Stability

The stability of the drug in solution during analysis was determined by repeated analysis of samples during the course of experimentation on the same day and also after storage of the drug solution for 48 hrs. under laboratory bench conditions ($33 \pm 1^\circ\text{C}$) and under refrigeration ($8 \pm 0.5^\circ\text{C}$).

Procedure for pharmaceutical formulation

For tablets, 20 units were weighed and finely powdered. An accurately weighed amount of the powder equivalent to 10 mg of Zolpidem tartrate was transferred into a 100 ml volumetric flask and sonicated for 15 min with 100 ml of mobile phase. The resulting suspension was filtered through 0.22 μm membrane filter. A suitable aliquot of this filtrate was diluted with mobile phase in order to obtain a final concentration of 10 to 60 $\mu\text{g mL}^{-1}$. A 10 μl of the obtained solution was chromatographed.

RESULTS AND DISCUSSION

Method Development

The HPLC procedure was optimized with a view to developing a method. From several solvents and solvent mixtures investigated acetonitrile and methanol (50:50, v/v) was found to furnish sharp, well-defined peak with very good symmetry and low t_R (3.46 min) (Fig. 1). Various other mobile phases tried earlier either did not give well defined peak in a short time, therefore were not considered. The final selection on mobile phase composition and flow rate was made on the basis of peak shape (peak area, peak asymmetry & tailing factor), baseline drift, time required for analysis, and cost of solvent, and acetonitrile and methanol (50:50 v/v) was selected as the optimum mobile phase. Under these conditions the retention time was 3.4 ± 0.01 min.

Validation of the Method

Linearity

The calibration plot of peak area against concentration was linear in the range investigated ($10\text{--}60 \mu\text{g mL}^{-1}$). The low values of RSD and standard error show the method is precise. Statistical calculations were performed at the 5% level of significance. The linear regression data for the calibration plot are indicative of a good linear relationship between peak area and concentration over a wide range. The linear regression equation was $y = 56009x + 40556$ and the regression coefficient was 0.999. The correlation coefficient was indicative of high significance. The low values of the standard deviation, the standard error of slope, and the intercept of the ordinate showed the calibration plot did not deviate from linearity. There were no significant differences between the slopes of standard curves constructed on different days.

Table 1: Optimized Chromatographic Conditions

Parameters	Conditions
Stationary phase (column)	HiQ Sil-C18 HS
Mobile Phase	Acetonitrile and Methanol (50:50 v/v)
Flow rate (mL min^{-1})	1.0
Runtime (min)	10
Column Temperature ($^\circ\text{C}$)	Ambient
Volume of Injection (μL)	20
Detection wavelength (nm)	293nm
Retention Time (min.)	3.45

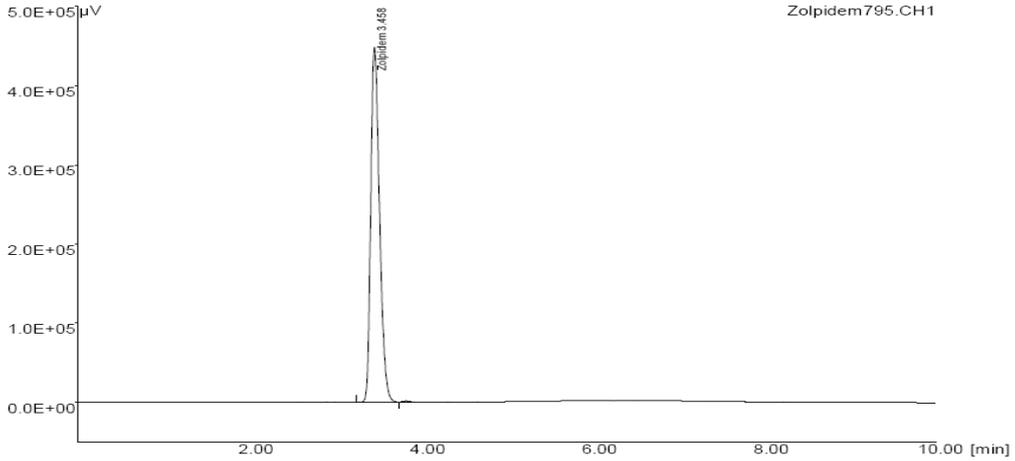


Fig. 2: A typical chromatogram of ZOL in acetonitrile and methanol (50:50, v/v) t_R 3.4 min

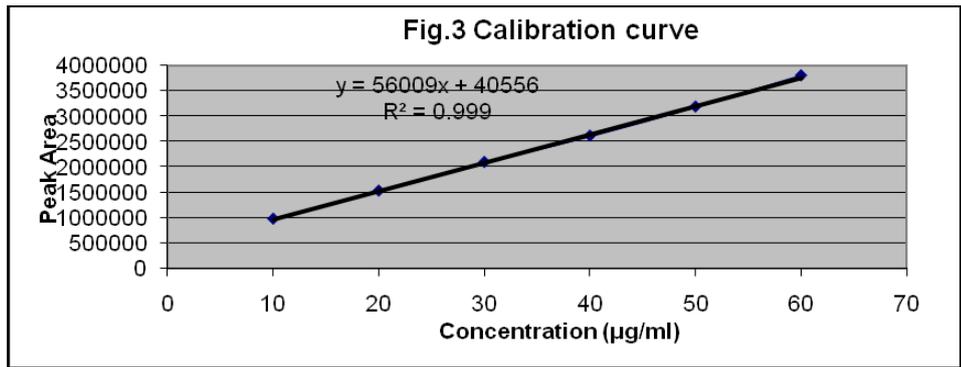


Fig. 3: Calibration curve

Table 2: Statistical data of calibration curves of ZOL

Parameters	ZOL
Linearity ($\mu\text{g mL}^{-1}$)	10 - 60
Regression equation	$56009x + 40556$
Correlation coefficient (R^2)	0.999

Table 3: System Suitability Parameters

Parameters	Obtained Values
Theoretical plates (N)	5799
Tailing Factor	0.08
LOD ($\mu\text{g mL}^{-1}$)	0.026
LOQ ($\mu\text{g mL}^{-1}$)	0.056

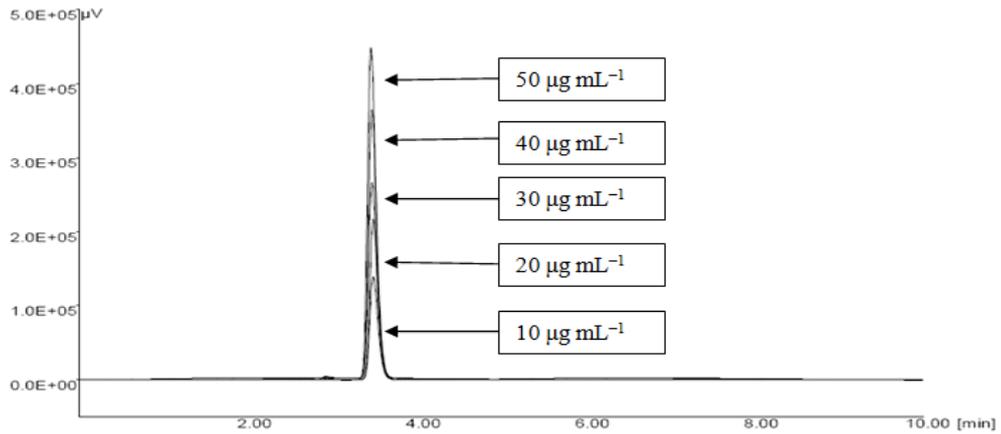


Fig. 4: A overlain chromatogram of ZOL

Accuracy

The recovery of the method, determined by spiking a previously analyzed test solution with additional drug standard solution, was 99.06–100.60%. The values of recovery (%), RSD (%), indicate the method is accurate.

Precision

Precision was considered at two levels, i.e. repeatability and intermediate precision, in accordance with ICH recommendations. Repeatability of sample injection was determined as intra-day variation whereas inter-mediate precision was determined by

measuring inter-day variation for triplicate determination of ZOL at single concentration (10 µg mL⁻¹). Results from determination of repeatability and intermediate precision, expressed as RSD (%). The low values of RSD indicate the repeatability of the method.

Reproducibility

Reproducibility was checked by measuring the precision of the method on another column with analysis performed by another person. Both intra-day and inter-day precision were determined. There were no significant differences between RSD (%) values for intra-day and inter-day precision, which indicates the method, is reproducible.

Table 4: Result of Recovery Studies of Zolpidem tartrate

Level of Recovery (%)	Amount Present in formulation (µg mL ⁻¹)	Amount of pure drug added (µg mL ⁻¹)	% Recovery*	R.S.D.	S.E.
80	10	8	99.06	1.49	0.027
100	10	10	100.48	0.95	0.038
120	10	12	100.60	1.68	0.035

*Indicates mean of three determinations, R.S.D. =Relative Standard Deviation, S.E. =Standard Error

Table 5: Result Analysis of Precision Studies

Concentration (µg mL ⁻¹)	Repeatability (intra day precision)*		Intermediate precision (inter day)*	
	% RSD	SE	% RSD	SE
10	0.68	0.58	0.61	0.85

* Indicates mean of six determinations, R.S.D. =relative Standard Deviation, S.E. =Standard Error

Table 6: Robustness of the method

System suitability parameters	Normal condition	Change in condition	Change in % RSD
Flow Rate	1.0 mL min ⁻¹	0.9 mL min ⁻¹ 1.1 mL min ⁻¹	0.031 0.028
Mobile phase ratio (Acetonitrile: Methanol)	50:50	46:54 54:46	0.046 0.038

Table 7: Results of the stress degradation tests using different conditions

Stress Condition	Solvent	Temp.	Time	% of ZOL
Acidic	1 M HCl	R.T.	48 h	95.2
Alkaline	1 M NaOH	R.T.	48 h	94.1
Oxidative	3% H ₂ O ₂	R.T.	48 h	57.4
Heat	Solid form	80 °C	5 days	96.0
	Mobile Phase	80 °C	5 days	95.9
Photolytic-UV light	Solid form	R.T.	48 h	94.2
	Mobile Phase	R.T.	48 h	94.0
Visible light	Solid form	R.T.	48 h	89.7
Visible light	Mobile Phase	R.T.	48 h	88.4

R.T. = Room temperature

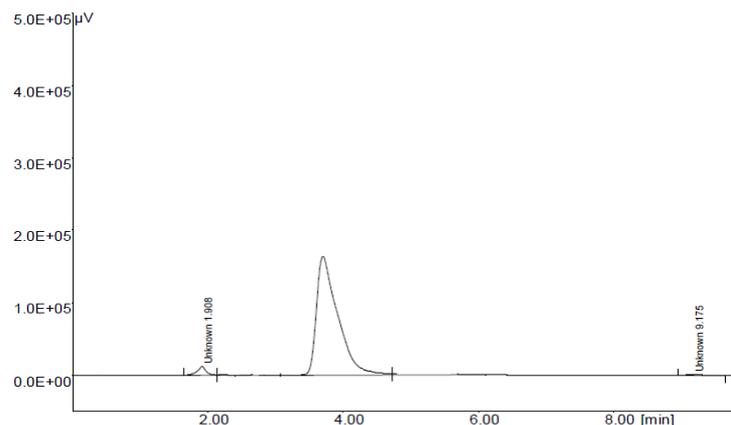


Fig. 5: Chromatogram of acid hydrolysis degradation.

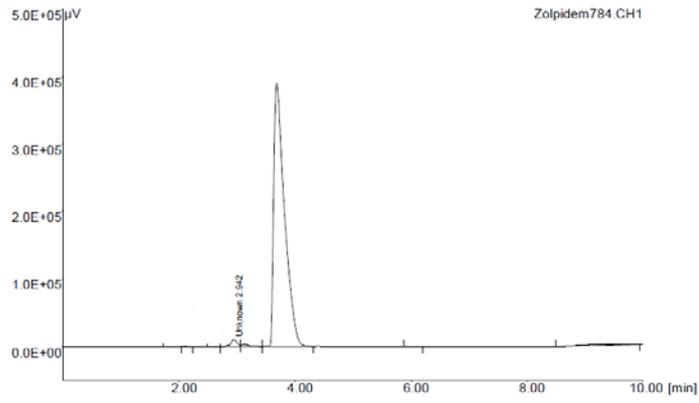


Fig. 6: Chromatogram of base hydrolysis degradation.

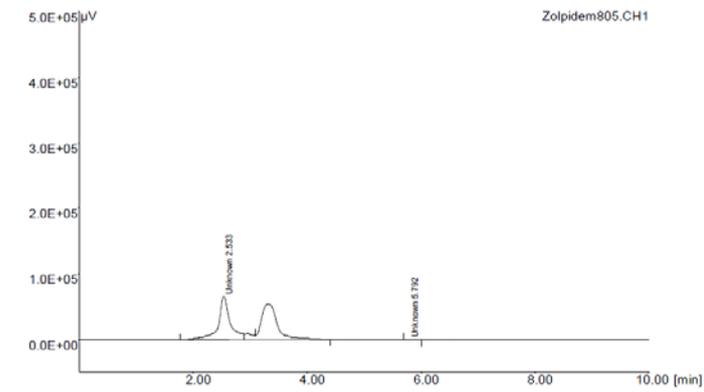


Fig. 7: Chromatograms of oxidative degradation

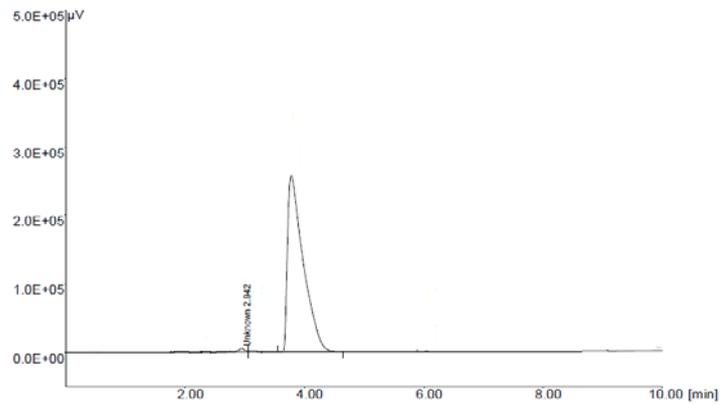


Fig. 8: Chromatograms of heat degradation product (80°C).

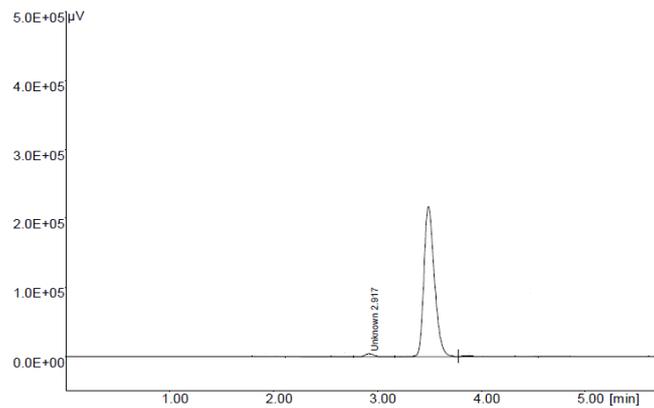


Fig. 9: Chromatograms of UV light degradation product.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD and LOQ of the method, determined by the standard deviation method, as described above, were 0.026 and 0.051 $\mu\text{g mL}^{-1}$, respectively, which indicated the method can be used for detection and quantification of ZOL over a very wide range of concentrations.

Robustness

There was no significant change in the retention time of ZOL when the composition and flow rate of the mobile phase were changed. The low values of the RSD indicated the robustness of the method.

Forced Degradation Study

Although conditions used for forced degradation were attenuated to achieve degradation in the range of 20–80%, this could not be achieved in all degradation conditions even after exposure for prolonged duration. Table 7 indicates the extent of degradation of ZOL under various stress conditions.

Typical chromatograms of degradation product are shown from Fig. 5 to 9. Concentration of ZOL decreases on acid and alkali hydrolysis,

forming a additional peak, considerable degradation was observed with oxidative degradation, drug was relatively stable on exposure to UV radiation. No other co-eluting peaks were found with the mean peaks suggested the specificity of the method for the simultaneous estimation of ZOL in presence of degradation products.

Stability

The drug was stable when stored for 48 hrs. at laboratory temperature ($32 \pm 1^\circ\text{C}$) and under refrigeration ($8 \pm 0.5^\circ\text{C}$) in acetonitrile and methanol (50:50 v/v).

Analysis of ZOL from tablet formulation

The proposed method was applied to the determination of Zolpidem tartrate in tablets formulation (10 mg). The mean average (three replicates) was found to be 9.98 mg corresponding to a mean recovery of 99.08% with an R.S.D. of 0.025%. This result was in good agreement with the label value. It should be pointed out that the chromatogram of the solution of excipients is absolutely free of any peak indicating thus that no interference from the excipients is encountered.

Table 8: Analysis of commercial formulation

Commercial formulation	Label claim (mg)	% Label claim estimated*	S.D.	%RSD
Tablet	10	99.08	0.048	0.025

SD= Standard deviation, RSD = Relative standard deviation, *Average of six determinations

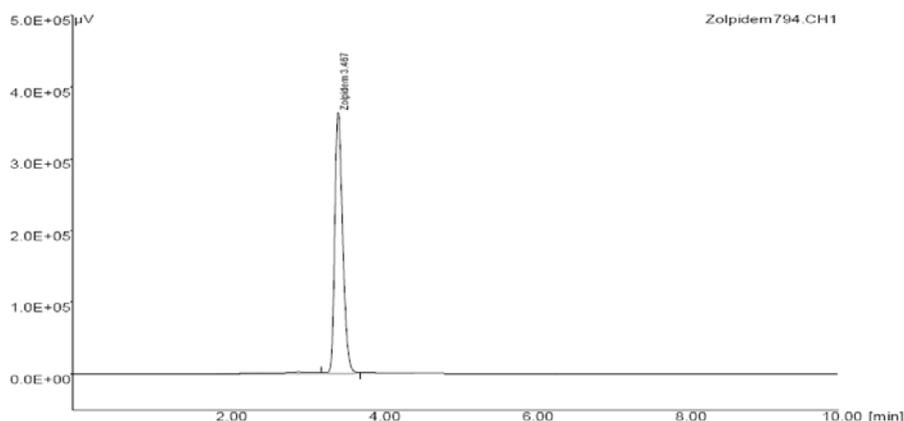


Fig. 10: A chromatogram of ZOL tablet formulation

CONCLUSION

A simple and rapid HPLC stability indicating method has been developed for the determination of Zolpidem tartrate. Statistical analysis of the results has been carried out revealing high accuracy and good precision. The method is reliable and convenient for routine control and stability assays of Zolpidem tartrate in both raw material and tablets.

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