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Research Article

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Stability-indicating RP-HPLC Method for the determination of Zolpidem tartrate and identification of its degradation products

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ABSTRACT

A stability-indicating, reverse phase high performance liquid chromatographic method for Zolpidem tartrate was developed after forced degradation, the formed degradants were separated on a Enable C_{18} column with a 35:65% v/v mixture of water containing 0.2% (v/v) triethylamine, (pH was adjusted to 7 with ortho phosphoric acid (OPA) and methanol as mobile phase. The flow rate kept at 1 mLmin−1 and the column effluents were detected at 243 nm. A sharp peak was obtained for Zolpidem tartrate at a retention time of 10.374 min. Forced degradation studies was carried out under acidic, basic, thermal, photolytic and oxidative conditions. Chromatographic peak purity was confirmed as no co-eluting peaks was obtained with the main peaks. One major degradation products from basic hydrolysis was identified and characterized by ^{1}H-NMR, FTIR and mass spectral data. The method was validated as per International Conference on Harmonization guidelines (Q2). Linear regression analysis data for the calibration plot showed good linear relationship between responses in the concentration range of 5 to 25 µg mL^{-1} with regression coefficient of 0.997. The relative standard deviations for intra and interday precision were below 2%. The formed degradation products was found to be non mutagenic towards Salmonella typhimurium strain TA98 and TA100.

Key words: Zolpidem tartrate, Stability studies, RP-HPLC, Identification, Mutagenicity.

INTRODUCTION

Zolpidem tartrate is chemically *N*, *N*-dimethyl-2-[6-methyl-2-(4-methylphenyl) imidazo [1, 2-*a*] pyridin-3-yl] acetamide L (+) – tartrate [1] (Fig.1). It is a short acting non benzodiazepine hypnotic of imidazopyridine class [2] that potentiates GABA, an inhibitory neurotransmitter, by binding to GABA receptors at the same location of benzodiazepines [3]. Its absorption and elimination is rapid [4]. A detailed literature survey reveals that UV Spectrophotometric methods [5-10], RP-HPLC [11-20], LC-MS[21], LC-MS/MS[22-23], GCMS[24] and electrophoresis methods [25-26] were reported for estimation of zolpidem tartrate. Degradation studies/characterization of the degradation products under stress conditions was not reported and hence the present study was undertaken to develop a new stability-indicating RP-HPLC method for the estimation of zolpidem tartrate and to identify the major degradants and to determine whether the formed degradants are mutagenic or not.

EXPERIMENTAL SECTION

Chemicals and reagent

Zolpidem tartrate was obtained as gift sample from M/s Orchid Pharmaceuticals, Chennai, India. HPLC grade methanol was purchased from Himedia Chemicals, India. Other chemicals and reagents were of analytical grade.

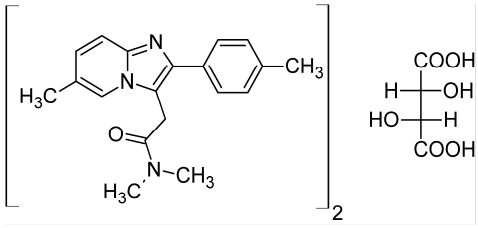


Fig.1 Structure of Zolpidem tartrate

Instrumentation and analytical conditions

Chromatographic analysis of Zolpidem tartrate was performed, under ambient conditions, with UFLC (Shimadzu, Japan) equipment comprising of an LC-20 AD isocratic pumps, manual injector with loop volume of 20 µl and a photo diode array detector and running on LC solution Software version. Compounds were separated on a 25 cm × 4.6 mm i.d., 5-µm particle, Enable C_{18} reverse phase column; with 35:65 (v/v) of 0.2% v/v triethylamine in water (pH 7 was adjusted using ortho phosphoric acid): methanol as the mobile phase, at a flow rate of 1.0 ml/min. The eluate was monitored at 243 nm, run time was 15 min and retention time was 10.374 min. The mobile phase was filtered through a 0.45 µm filter paper and sonicated before use. A preparative HPLC equipped with Enable C_{18} column (250x20mm, 10µ) and PDA detection (using the software lab solution) was used for the isolation of degradation products. ^{1}H-NMR was recorded on the Varian Unity Inova at 400 MHz (using TMS as internal standard and DMSO-d_6 as solvent), IR was recorded on FTIR (JASCO 4100) and mass spectral studies were performed on API 3000 ABPCIES instrument.

Forced Degradation Studies

Forced degradation of Zolpidem tartrate was performed under acid, base, oxidative, thermal and photolytic stress conditions. In all stress conditions, the drug concentration used was 1000 µgmL−1. After degradation, samples were diluted with mobile phase to a concentration of 10 µgmL−1 and injected under optimized conditions with the appropriate blank.

Preparation of Stock Solution for Stress Studies

Ten mg of working standard of Zolpidem tartrate was accurately weighed and dissolved in 10ml of methanol to give a stock solution of 1mg/ml.

Acid hydrolysis

Acid-induced, forced degradation was performed by adding an aliquot of stock solution (1 mg/ml) of Zolpidem tartrate to 10 ml of 1 M HCl and refluxing the mixture at 80°C for four hours. The solution was then left to reach room temperature, neutralized to pH 7 by the addition of 1 M NaOH, and diluted with the mobile phase to get a final concentration of10 µg/ml.

Alkaline hydrolysis

Forced degradation in alkaline media was performed by adding an aliquot of stock solution (1 mg/ml) of Zolpidem tartrate to 1 M NaOH, and refluxing the mixture at 80°C for three hours. The solution was then left to reach room temperature, neutralized to pH 7 by addition of 1 M HCl, and diluted with the mobile phase to get a final concentration of 10 µg/ml.

Oxidative degradation

To study the effect of oxidizing conditions, an aliquot of stock solution (1 mg/ml) of Zolpidem tartrate was added to 10 ml of 3% H_2O_2 solution and the mixture was refluxed at 80°C for three hours. The solution was left to reach room temperature and diluted with the mobile phase to get a final concentration of 10 µg/ml.

Thermal degradation

To study the effect of temperature, approximately 100 mg Zolpidem tartrate was stored at 85°C in a hot air oven for 1 week. 10 mg of the heated sample was weighed, dissolved in methanol, diluted with mobile phase to 10 µgmL−1 and analyzed.

Photolysis

To study the effect of UV light, approximately 100 mg of Zolpidem tartrate was exposed to short and long wavelength UV light (254 nm and 366 nm, respectively) for 1 week.10mg of the sample was dissolved in methanol, diluted with the mobile phase to give a solution of final concentration equivalent to 10 µg/ml of zolpidem tartrate.

In all of the degradation studies, the percentage degradation of Zolpidem tartrate was calculated. The formed degradation product was isolated on a Enable C_{18} column (250x20mm, 10µ) by using preparative HPLC and then evaporated in a rotary vacuum evaporator. One degradation product from basic hydrolysis was isolated and characterized by FTIR, ^{1}H-NMR and mass spectrometry.

Mutagenicity [27]

Pre incubation method was done for mutagenicity assay. Different concentration of degradants is preincubated with the test strain and sterile buffer for 20 min at 30-37^{0}C and mixed with overlay agar, immediately transfer onto the surface of a minimal agar plate. Plates were incubated for 48 h at 37°C and the relevant colonies were counted.

RESULTS AND DISCUSSION

Method Development and Optimization of the Chromatographic Conditions

The stability-indicating ability of the method was thus established. Maximum degradation of zolpidem tartrate was observed in alkaline medium, followed by decomposition under acid, oxidative, thermal degradation and photolysis. Percent degradation of zolpidem tartrate under all stressed conditions was included in [Table 1]. Separation of zolpidem tartrate from its degradation products has been performed on an RP C_{18} column (250 x 4.6 mm, 5µ). Initially water and methanol in various proportions were tried. However, good resolution between peaks was not obtained. Different ratios of triethylamine in water (pH 7) and methanol were tried. In order to ensure complete separation and high resolution, the chosen ratio was 35: 65 (% v/v). Simultaneous monitoring with the PDA detector was carried out at a range of wavelength between 200 – 400 nm. Detection was carried out at 243 nm, where maximum sensitivity was observed. The chromatogram obtained in the optimized condition is shown in [Fig 2]. One degradation product was formed with retention times 3.91 for base (1M NaOH) hydrolysis. The chromatographic resolution among all of the peaks was found to be more than 2. The specificity of the method is illustrated in the chromatograms of the degradation studies.

Table 1: Degradation data of Zolpidem tartrate under stress studies

Degradation studies	Time	Assay of Zolpidem tartrate (% of peak area)	Remarks
Acid hydrolysis	4h	95.18	Degradation observed
Base hydrolysis	3h	80.66	Degradation product 1 observed
Oxidation	3h	97.09	Degradation observed
Photolytic condition	1 week	96.17	Degradation observed
Thermal condition	1 week	92.30	Degradation observed

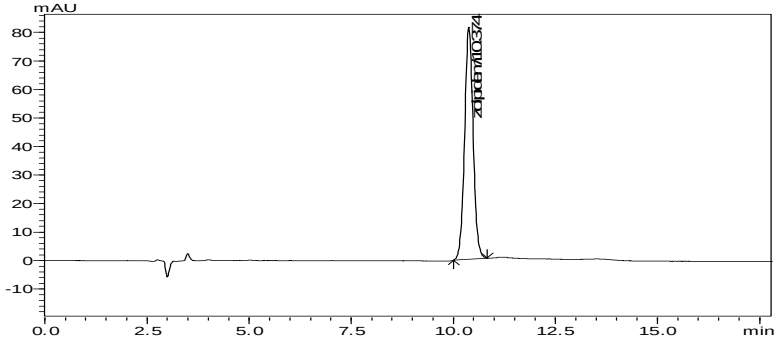


Fig.2 Chromatogram of Zolpidem tartrate (10µg/ml)

Validation Parameters

The method was validated as per ICH (Q2) guidelines with respect to linearity, accuracy, precision, robustness, limit of detection and limit of quantification [28].

Specificity

Specificity of the method towards the drug was studied by determination of purity for drug peak in stressed sample using a PDA detector. The study of resolution factor of the drug peak from the nearest resolving degradation product (DP) was also done. The resolution factor for the drug from degradation peak was greater than 2. The method was proven to be specific by separating the degradation products formed under the stress conditions.

Linearity

Five points graph was constructed covering a concentration range 5 to 25 µgmL^{-1}. Linear relationships between the peak area and concentration of Zolpidem tartrate was recorded and evaluation was performed and calibration curve was shown in [Fig 3]

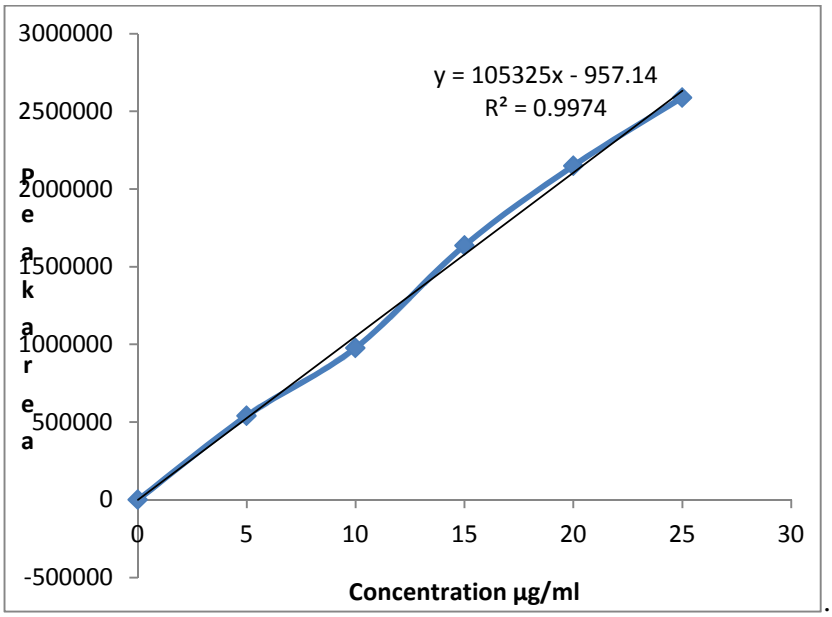


Fig.3 Calibration curve for Zolpidem tartrate

Intraday and Interday precision

Intraday precision was found out by carrying out the analysis of the Zolpidem tartrate in the linearity range for six times on the same day. Inter day precision was found out by carrying out the analysis of the Zolpidem tartrate in the linearity range for three days for six times and Percentage RSD was calculated which is found to be within acceptable criteria of not more than 2 [Table 2]. Low values of standard deviation denoted very good repeatability of the measurement. Thus it was showing that the equipment used for the study was correctly and hence the developed analytical method is highly repetitive.

Table 2: Intra-day and inter-day precision of the developed method

Concentration (µgmL^{-1})	Measured concentration (µgmL^{-1})			
	Intraday(n=6)	%RSD	Interday (n=3)	%RSD
10	9.99	1.553	9.83	0.107
15	15.24	1.873	15.04	1.239
20	19.95	0.764	20.02	1.559

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

The LOD and LOQ of the method, determined by the standard deviation method, which indicated the method, can be used for detection and quantification of Zolpidem tartrate over a very wide range of concentrations.

Accuracy

The recovery of the method, determined by spiking a previously analyzed solution with additional standard Zolpidem tartrate solution. The values of recovery (%), RSD (%) in [Table 3] indicated that the method is accurate.

Table 3: Accuracy (Recovery studies) results of the developed method

Concentration (µgmL^{-1})	Spike Level (%)	Percentage Recovery	% RSD*
10	50	101.54	1.461
	100	100	1.300
	150	99.28	0.497

**average of 5 determination*

Robustness

There was no significant change in the retention time of Zolpidem tartrate with the change in flow rate and composition of the mobile phase. Percentage RSD was calculated which is found to be within acceptable criteria of not more than2.The low values of the RSD indicated that the method is robust.

System suitability studies

The statistical evaluation of the proposed method was revealed its good linearity, reproducibility and its validation for different parameters. The results are furnished in [Table 4].The detail degradations of Zolpidem tartrate is shown in [Table 1] and chromatogram are shown from [Figure 4 to 8]. The degradation product was adequately separated, thus the method was found to be more selective and specific. The peak purity was more than 0.997. The study revealed that Zolpidem tartrate was more sensitive to all stress conditions. Zolpidem tartrate was more labile to degradation in the presence of base.

Table 4: System Suitability studies

S No.	Parameters	Zolpidem tartrate
1	Theoretical plate	10949
2	HETP	13.69
3	Tailing factor	1.086
4	LOD	0.568
5	LOQ	1.723

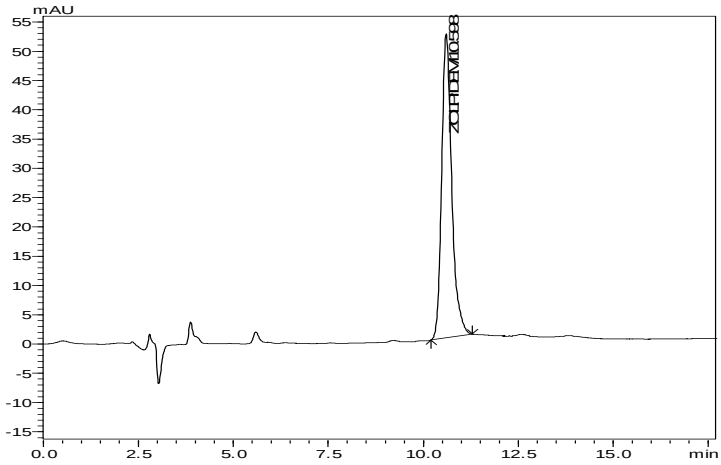


Fig.4 Chromatogram of Zolpidem tartrate after degradation with 1M HCl

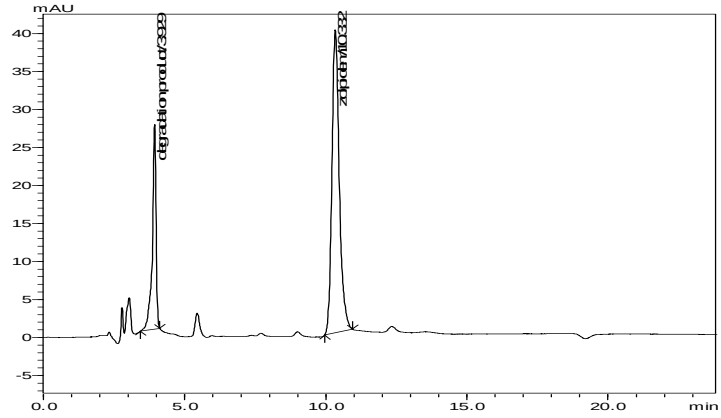


Fig.5 Chromatogram of Zolpidem tartrate after degradation with 1M NaOH

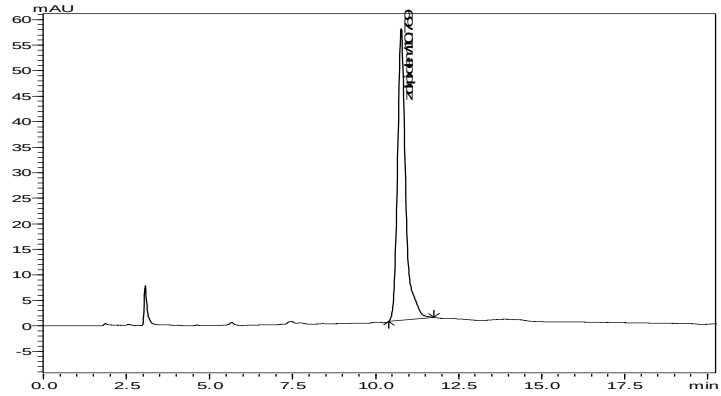


Fig.6 Chromatogram of Zolpidem tartrate after degradation with Peroxide (3%)

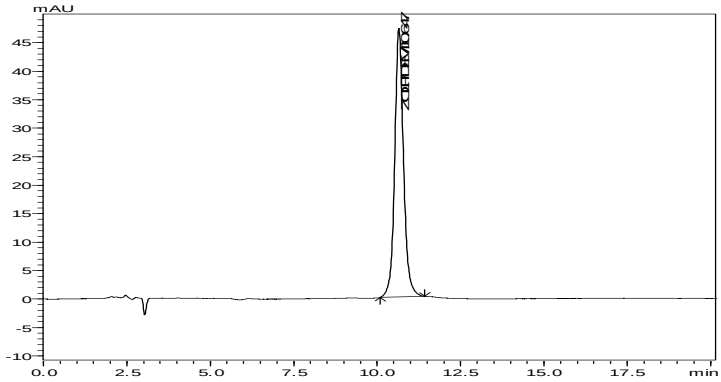


Fig.7 Chromatogram of Zolpidem tartrate after thermal degradation

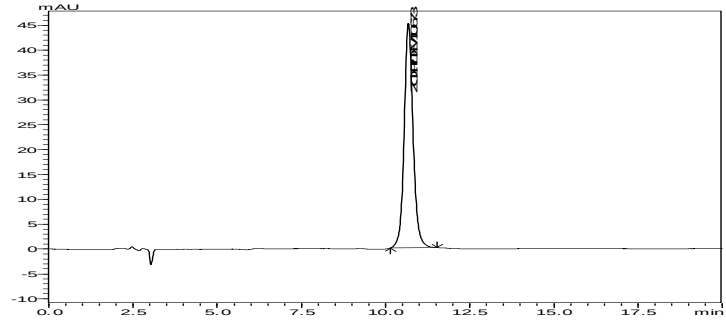
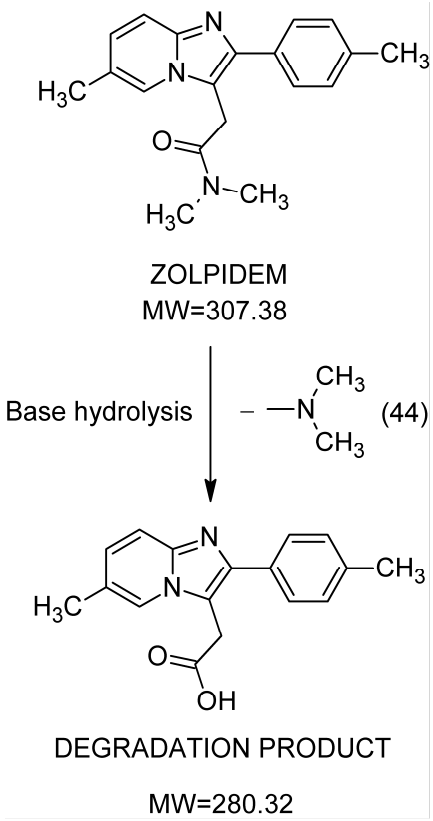


Fig.8 Chromatogram of Zolpidem tartrate after Photolytic degradation

Identification of Degradants

In these forced degradation studies, one degradation product was observed for Zolpidem tartrate, (basic hydrolysis), separated by preparative HPLC chromatography. The obtained degradation products were characterized by FTIR, ^{1}H-NMR, and mass spectral data. The retention time for degradation product was identified as 3.91 min for base hydrolysis.

Degradant1: [2-(4-hydroxy phenyl)-6-methylimidazo [1,2-a]pyridine-1-yl]acetic acid. Formula: $C_{17}H_{16}N_2O_2$, molecular weight: 280, ^{1}H-NMR (CDCl3, δ ppm): 3.165 (s, 2H, CH_2), 8.194 (s, 1H, Ar H), 4. (s, 1H, OH), 2.510(s, 2H, CH_2). IR (KBr): 3440(OH), 1410 (CH3), 2921(CH_2), 1638(C=O). The probable structure of degradation product is given in scheme 1.The formed degradation product did not induce any mutations in TA 98, TA 100 *S. typhimurium* strains.



Scheme.1 Possible mechanism of the degradation of Zolpidem tartrate

CONCLUSION

Forced degradation studies on Zolpidem tartrate carried out according to ICH guidelines and it was found to be susceptible to basic hydrolytic degradation. Degradation product was separated in a single run by an isocratic LC-DAD method. The method proved to be simple, accurate, precise, specific and robust. The degradation product was identified as [2-(4-hydroxy phenyl)-6-methylimidazo [1, 2-a] pyridine-1-yl] acetic acid using IR, NMR and mass spectral data and it was found not mutagenic in Ames test

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