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Stability-indicating HPLC method for determination of zolpidem in pharmaceutical dosage form

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ABSTRACT

The objective of the method was to develop a simple, precise, rapid and stability indicating high performance liquid chromatographic method (HPLC) for the quantitative determination of a short acting hypnotic drug zolpidem in its formulations. In this method Hypersil-100 ODS-3 column (Thermo Scientific, Waltham, USA) [C_{18} (5 µ), 25 cm X 4.6 mm, i.d)] was used at ambient temperature using mobile phase consisting of acetonitrile: 30mM phosphate buffer (60: 40 v/v) pH adjusted to 3.5 ± 0.02 with ortho phosphoric acid gave a symmetrical, sharp peak at ($t_{R=}$ 7.02 ± 0.05 min). The detection wavelength was 242 nm and the flow rate 1 mL min^{-1}.In the range of 1 - 6 µg mL^{-1} the linearity of zolpidem showed a regression coefficient of 0.999. The proposed method was sufficiently selective to distinguish the parent drug and the degradation products after hydrolytic, oxidative, dry heat, wet heat, neutral and photochemical degradation products and from excipients. This developed method was validated by determining its sensitivity, accuracy and precision. The developed method was simple, fast, accurate, precise which separates the drug from its degradants and hence could be applied as a stability indicating method for routine quality control analysis of zolpidem in dosage forms.

Key words: Zolpidem, HPLC, Degradation, Validation, Stability Indicating.

INTRODUCTION

Zolpidem chemically is, N,N,6-trimethyl-2-(4-methylphenyl)-imidazo (1,2-a) pyridine-3-acetamide (Fig.1) , and is a short-acting nonbenzodiazepine hypnotic that potentiates gamma-aminobutyric acid (GABA), an inhibitory neurotransmitter, by binding to benzodiazepine receptors which are located on the gamma-aminobutyric acid receptors. Its hypnotic effects are similar to those of the benzodiazepine class of drugs, but it is molecularly distinct from the classical benzodiazepine molecule and is actually classified as an imidazopyridine. Flumazenil, a benzodiazepine receptor antagonist, which is used for benzodiazepine overdose, can also reverse zolpidem's sedative/hypnotic effects. As an anticonvulsant and muscle relaxant, the beneficial effects start to emerge at 10 and 20 times the dose required for sedation, respectively. For that reason, it has never been approved for either muscle relaxation or seizure prevention [1-2].

Literature survey reveals, selective potentiometric determination of zolpidem hemitartrate in tablets and biological fluids by using polymeric membrane electrodes, determination of zolpidem in human plasma using LC with fluorescence detection, LC-tandem mass spectrometry method for the simultaneous determination of 26

benzodiazepines and metabolites, zolpidem and zopiclone, in blood, urine, and hair, determination and in-process control of zolpidem synthesis by LC, Rapid and simple method for the determination of zolpidem in human plasma by high-performance liquid chromatography and determination of zolpidem hemitartrate by quantitative HPTLC and LC have been reported [3-7].

Quantitative estimation of the degradants in reported methods was done by HTLC and LC, but the research article emphasized on its degradant separation only in acidic conditions and the validation was done with few parameters. The present proposed research work is done only by LC and the degradation studies include alkaline, oxidative, hydrolytic, dry, wet heat, neutral hydrolysis and photolytic conditions. The developed method is further validated as per ICH guideline and has proved to be robust. Hence it was thought to develop an additional stability indicating study in all conditions. The proposed methods have been applied successfully to tablets containing the drug which separates the degradants completely from the parent peak [8-14].

The present manuscript describes validated, simple, sensitive, accurate, precise and specific stability indicating HPLC procedure for the determination of zolpidem in pharmaceutical dosage forms.

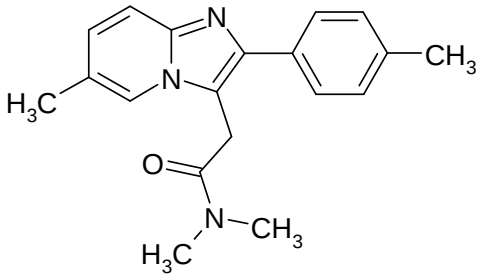

Figure 1: Chemical structure of zolpidem

MATERIALS AND METHODS

2.1. Chemicals and Reagents

Pharmaceutical grade zolpidem was kindly supplied as a gift sample by Shreeji Pharma International, Vadodara, India. It was certified to contain 99.85% (w/w) on dry basis and was used further without purification. Acetonitrile of HPLC grade was purchased from Merck Chemicals, India. All other chemicals and reagents used were of analytical grade and were supplied from Merck Chemicals, India.

2.2. Instrumentation

The HPLC system (Jasco corporation, Tokyo, Japan) consisted of a Pump (model Jasco PU- 2080 Plus) along with manual injector sampler programmed at 20 µL capacity per injection was used. The detector consisted of UV/ VIS (model Jasco UV 2075). LC separations were performed on a Hypersil-100 ODS-3 column (Thermo Scientific, Waltham, USA) [C_{18} (5 µ), 25 cm X 4.6 mm, i.d)]. Data was integrated using Jasco Borwin version 1.5, LC-Net II/ADC system. The mobile phase consisted of a mixture of acetonitrile: 30mM phosphate buffer (60: 40 v/v); pH adjusted to 3.5 ± 0.02 with ortho phosphoric acid gave a symmetrical, sharp peak at ($t_{R=}$ 7.02 ± 0.05 min^{-1}). The mobile phase was degassed and filtered by passing through a 0.45 μm^{-1} pore size membrane filter (Millipore, Milford, MA, USA) prior to use. The flow rate was 1 mL min^{-1}. All determinations were performed at ambient temperature with a detection wavelength of 242 nm.

2.3. HPTLC Method and Chromatographic Conditions

2.3.1.Preparation of Standard Solution

Standard stock solution containing 1 mg ml^{-1} of zolpidem was prepared in acetonitrile. The working standard was prepared by diluting the above stock solution in mobile phase to reach a concentration range of 1-10 µg ml^{-1}.

2.3.2. Preparation of Sample Solutions

The pharmaceutical dosage form used in this study was zoldem, manufactured by Consern Pharma Private Ltd. (Ludhiana, Punjab) labelled to contain 5 mg of zolpidem per tablet. From the powdered mass of 10 tablets, an amount equivalent to 5 mg of zolpidem was weighed, transferred to a 50 mL volumetric flask, and dissolved in 25 mL of mobile phase. The solutions were sonicated for 30 min to enable dissolution of the active components from

tablets and then diluted to volume with the mobile phase to obtain sample solutions containing 0.10 mg ml^{-1} of zolpidem. After mixing, the solutions were filtered through whatmann filter paper No. 0.45 µm. The analysis was performed three times. The possibility of excipients interference with the analysis was examined.

2.4. VALIDATION OF THE METHOD

2.4.1. Specificity

The specificity of the method was tested by comparing the chromatograms of standard zoldem with that of sample. Marketed formulation was processed and analyzed under the same conditions, and the retention times of zoldpidem standard and zoldem from marketed formulation were detected.

2.4.2. Calibration Curve

Six standard samples of zolpidem (1-6 µg ml^{-1}) were prepared to generate the calibration curve. To avoid bias, standard curves were fitted using weighted least squares linear regression in the form of y = a + bx, where y represents the ratio of zolpidem peak area and x represents the concentration of zolpidem. Calibration curves were prepared and analyzed on three consecutive days to evaluate the linearity.

2.4.3. Accuracy, Precision and Limit of Quantification

The accuracy and precision of the method were evaluated with 80, 100 and 120 % of the test concentration as per ICH guidelines (n = 3). Accuracy was obtained by calculating the ratios of the concentrations calculated with the calibration curves to the spiked values, and expressed as percentages. To validate the intraday and interday precision at three levels were freshly prepared and determined by quantitating three replicates on the same day and three consecutive days, respectively. The precision was calculated as the coefficient of variation (CV) of measurements.
The limit of quantification (LOQ) was estimated by analyzing the low concentrations of zolpidem in the calibration curves which could be quantitated accurately and the LOQ was verified by the analysis of three replicates.

2.4.4. Robustness

The robustness is a measure of method capacity to remain unaffected by small but deliberate changes in chromatographic conditions. It was studied by testing influence of small changes in flow rate (± 0.1mL min^{-1}), change in mobile phase composition acetonitrile ± 1 mL.

2.5. Accelerated degradation of fexofenadine hydrochloride

Accelerated degradation was carried by exposing the drug to different stressed conditions.

A drug stock solution of zolpidem (1mg) was prepared in 10 mL acetonitrile. This drug solution was used for forced degradation to provide an indication of the stability indicating property and specificity of the proposed method.

DEGRADATION STUDIES

2.5.1. Acid and Base Induced Degradation

Acid decomposition studies were performed by exposing the solution of drug to 5 N hydrochloric acid refluxed at 80°C for 8 h The studies in alkaline conditions were carried out in 5 N sodium hydroxide and the solution was refluxed at 80°C for 8 h The resultant solutions were diluted in mobile phase and the sample solution was then injected separately under the optimized chromatographic conditions used for analysis of marketed formulation.

2.5.2. Hydrogen Peroxide Induced Degradation

To study hydrogen peroxide induced degradation, the sample was exposed to 50% hydrogen peroxide and the solution was refluxed at 80°C for 8 h Resultant solution was diluted in mobile phase and then injected separately under the optimized chromatographic conditions used for the analysis of marketed formulation.

2.5.3. Dry and wet heat degradation

The powdered drug was stored in oven at 100°C for 10 h to study dry heat degradation and the stock solution was refluxed for 10 h for wet heat degradation

2.5.4. Neutral hydrolysis:

To 20 mL of stock solution, 20 mL of double distilled water was added and the mixture was refluxed for 12 h at 100°C to study the degradation under neutral conditions and was analyzed.

2.5.5. Photochemical Degradation

The photochemical stability of the drug was studied by exposing the stock solution (1000 µg mL^{-1}) to direct sunlight for 24 h. The resultant solution was diluted in mobile phase and injected separately under the optimized chromatographic conditions used for analysis of marketed formulation. The drug solution was also kept in the photostability chamber for 24 h. Appropriate dilutions of pure drugs were prepared in mobile phase and then were analyzed under the optimized chromatographic conditions.

RESULTS AND DISCUSSION

3.1. Optimization of HPLC Method

Quantitative HPTLC and LC method described by Zeany et al. [1] for the determination of zolpidem hemitartrate resolved the peak by using 40:60 acetonitrile: 0.01% NaH_2PO_4, pH was adjusted to 3.59 ± 0.1 with a flow rate of 1.2 mL min^{-1} and the retention time of was 6.7 min in presence of its acid degradants. The proposed work resolved the drug by using a mixture of acetonitrile: 30mM phosphate buffer (60: 40 v/v); pH adjusted to 3.5 ± 0.02 with ortho phosphoric acid with a flow rate of 1.0 mL min^{-1} which gave a symmetrical, sharp peak at retention time of 7.02 ± 0.05 min.

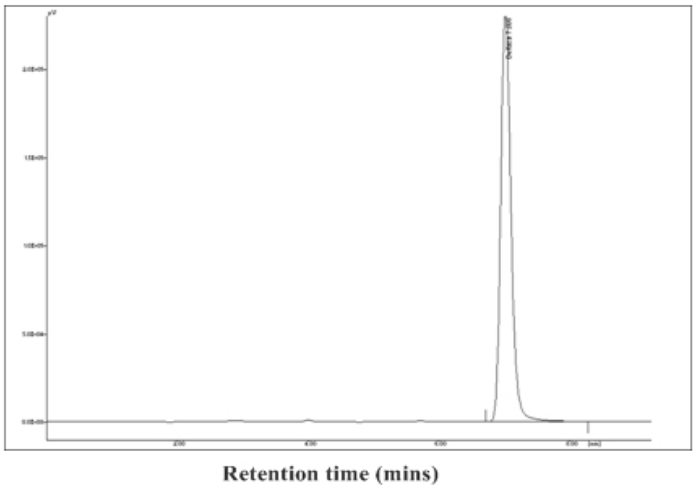

Figure 2: Chromatogram of zolpidem t_R: 7.02± 0.05

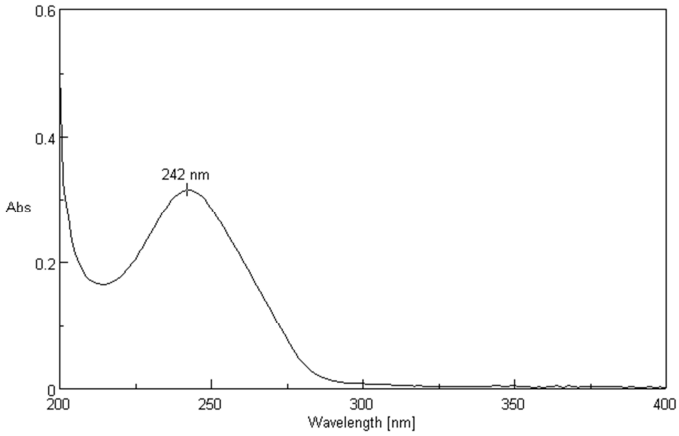

Figure 3: UV Spectra for Zolpidem

Chromatogram of zolpidem at a concentration of 10 µg ml^{-1} is shown in (Fig.2) Significant interference from endogenous substances was not observed at the retention times of zolpidem and marketed formulation, indicating

the sample preparation method is effective. The peaks were sharp and symmetrical with good baseline resolution and minimal tailing.

UV spectrum of zolpidem is shown in (Fig.3) showed maximum absorbance at 242 nm and the same was selected as the scanning wavelength. The mobile phase was optimized by changing the composition of the mobile phase to achieve good resolution and symmetric peak shapes for analyte as well as a short run time.

VALIDATION OF THE METHOD

3.2. Linearity and LOQ

The reported method determined the linearity in range of 0.5- 5 mg mL^{-1} with an LOD and LOQ of 0.035 and 0.106 µg mL^{-1} respectively. This proposed method determined LOD, LOQ by visual detection method and was found to be 0.1 and 0.4 µg mL^{-1} respectively after studying a range of concentrations made from the stock solution. The results of the linearity studies for calibration curve are depicted in Table 1 and (Fig.4.a & 4.b).

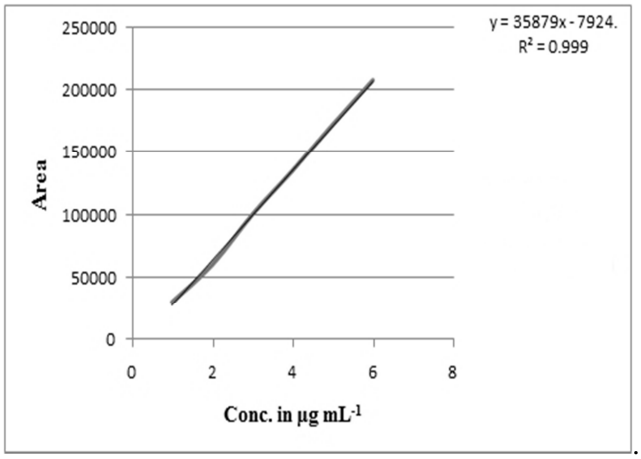

Figure 4.a: Calibration Curve of Zolpidem

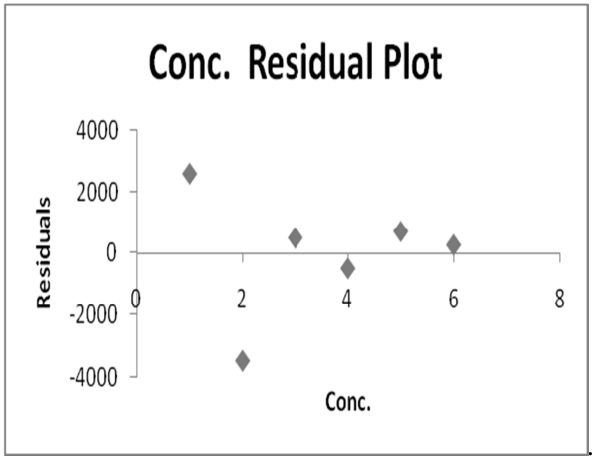

Figure 4.b: Concentration Residual Plot

3.2. Precision

The precision of the method was determined by analysis of the marketed formulation. The repeatability of sample injection and intermediate precision, as RSD were less than 2% for zolpidem indicating acceptable degree of intra and inter-day precision and the data is depicted in Table 2.

Table 1 Linearity Regression Data for Calibration Curve

Parameters	Zolpidem
Linearity range	1-6 µg mL^{-1}
r^2	0.999
Slope	38879 ± 530.1
Intercept	-7860
Confidence limit of slope [a]	34410 to 37350
Confidence limit of intercept [a]	13660 to 2194
Sy.x [b]	2218
P value [c]	< 0.0001

[a] *95% Confidence intervals*
[b] *Standard deviation of residuals from line*
[c] *P value is < 0.0001, considered extremely significant*

Table 2 Intra-Day and Inter-Day Precision of Zolpidem

Conc. mg mL-1	Intra-day precision (n=3)			Inter-day Precision (n=3)		
	Found conc. ± SD	RSD (%)	S.E.	Found conc. ± SD	RSD (%)	S.E.
2	1.95 ± 0.004	0.26	0.002	2.006 ± 0.003	1.49	0.0012
4	4.05 ± 0.012	0.29	0.004	4.100 ± 0.015	0.36	0.006
6	5.90 ± 0.09	1.53	0.036	5.90 ± 0.003	0.5	0.0012

3.3. Robustness

Reported method does not show the data of robustness. Hence the developed method is proved to be robust. To evaluate the robustness of the method, the optimized method parameters were varied at different levels. The results are presented in Table 3 which indicated that the developed method was unaffected by small variations in the optimized method parameters and was found to be robust.

Table 3 Robustness of the method

Level	Factor	Retention Time	% RSD
Change in Conc. of Acetonitrile in Mobile Phase (n=3)			
-1	59	7.29	1.51
0	60	7.02	1.13
1	61	7.2	1.26
Change in Flow Rate (n=3)			
-1	0.9	7	0.63
0	1	7.02	1.01
1	1.1	7.09	0.88

3.4. Accuracy

The reported method was found to be accurate with value of 99.96 ± 0.986 whereas the proposed method gave mean % recovery of 100.36 % for zolpidem. The accuracy was studied by measurement of recovery at three different levels (equivalent to 80, 100, and 120% of the amount originally present in the tablets). The data for accuracy studies is as depicted in Table 4.

Table 4 Accuracy study of the method

Drug	Label claim mg tab^{-1}	Amount added in mg (%)	Total amount (mg)	Amount Recovered (mg) ± SD	RSD (%)	Recovery (%)
Zolpidem	5	4.8 (80)	10.8	10.98±0.2	1.82	102.66
		5 (100)	12	11.78±0.09	0.76	98.16
		7 (120)	13.2	13.25±0.22	1.66	100.27

3.5. Specificity

The reported method does not show the data of specificity. For specificity studies the content of drug was analyzed in the tablets. The specificity of the methods is illustrated in (Fig.5) where complete separation of zolpidem in presence of its excipients was observed. The average retention time ± standard deviation for zolpidem was found to be 7.01 ± 0.05 min, for six replicates.

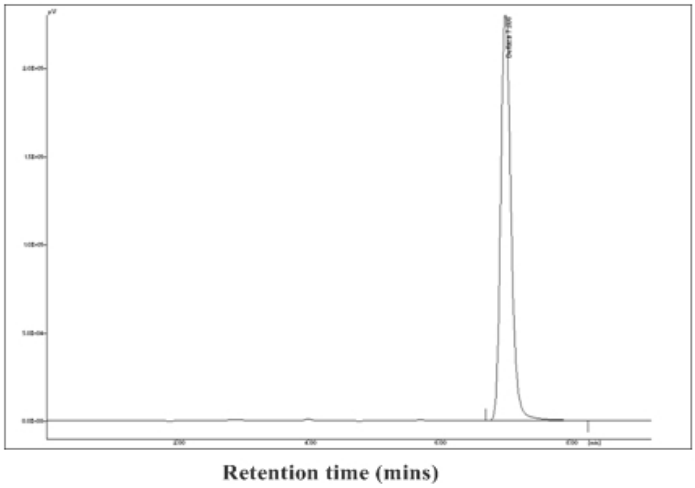

Figure 5: Chromatogram of Zoldem Tablet

FORCED DEGRADATION STUDIES

3.6.1. Acid and Base Induced Degradation

Reported methods show degradation pattern only in acidic conditions, the work does not include the degradation study widely. The present method shows degradation in alkaline conditions. Optimized condition for acid and base degradation was achieved by exposing the solution to 5 N hydrochloric acid and 5 N sodium hydroxide and the solutions were refluxed at 80°C for 8 h. The solution was then diluted with mobile phase and injected into the column. The chromatogram of the acid degraded sample showed one additional peak at t_R 5.70 min, (Fig.6.a) and in base degraded sample showed one additional peaks at t_R 3.01 min, (Fig.6.b)

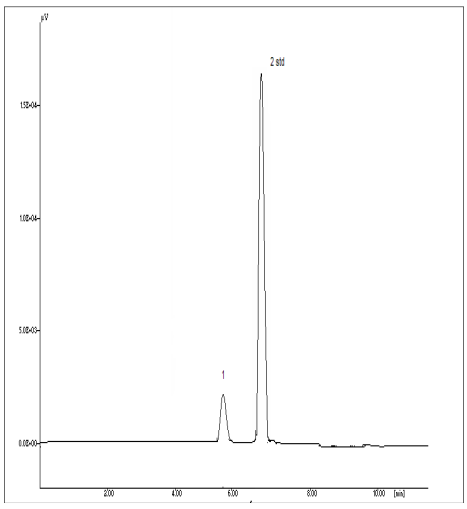

Figure 6.a: Chromatogram for acid induced degradation

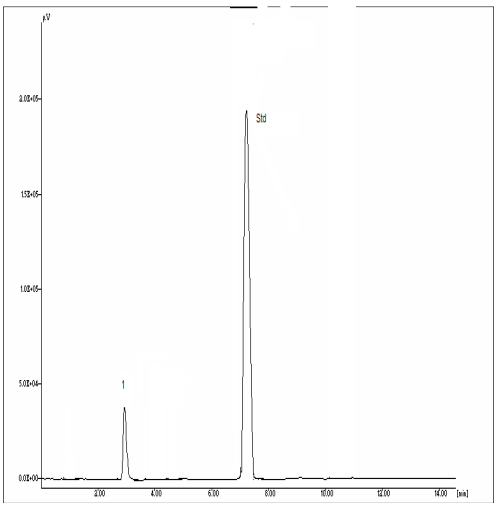

Figure 6.b: Chromatogram for base induced degradation

3.6.1. Hydrogen Peroxide Induced Degradation

The chromatogram of the hydrogen peroxide degraded sample showed one additional peak at t_R 8.21 (Fig.7).

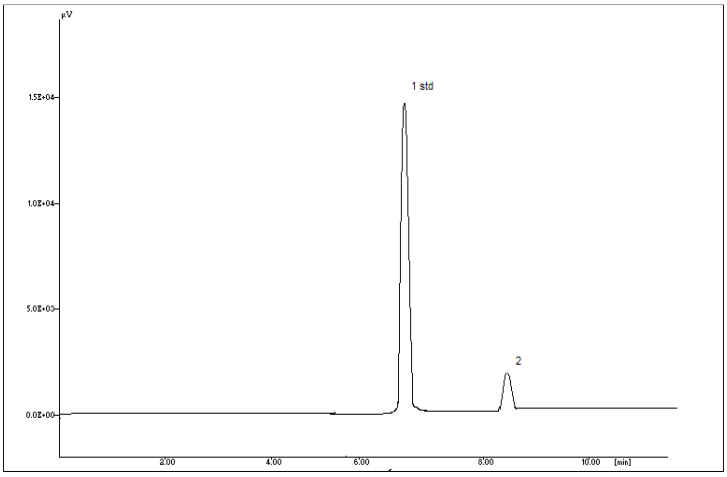

Figure 7: Chromatogram for hydrogen peroxide induced degradation

3.6.2. Dry and wet heat degradation

The samples degraded under wet heat and dry heat conditions showed no additional peaks

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3.6.3. Neutral degradation

The samples degraded in neutral hydrolysis showed no additional peaks.

3.6.4. Photochemical Degradation

The samples were kept in sunlight and showed no additional peaks.

The summary of degradation studies is depicted in Table 5, which proves that complete separation of the drug was achieved in presence of degradants which comply with the ICH guidelines provided.

Table 5 Summary of Degradation Studies

Conditions	Number of Peaks	Retention Time
Acidic (5.0 N HCl)	Peak 1	5.71
Alkaline (5.0 N NaOH)	Peak 1	3.02
Oxidation (50% H_2O_2)	Peak 1	8.21
Dry and Wet heat	--	---
Neutral	---	---
Photochemical	--	---

CONCLUSION

The proposed HPLC method is simple, accurate, reproducible and stability indicating for quantitative determination of zolpidem in bulk and in pharmaceutical formulation without interference from the excipients and in the presence of its acidic, alkaline and oxidative degradants. The developed method can be termed as validated stability indicating method in acidic, alkaline, oxidative, hydrolytic, photochemical conditions. The chromatographic method was validated as per the ICH guidelines. Statistical tests indicate that the proposed method reduces the duration of analysis and appears to be equally suitable for the routine analysis in QC laboratories as compared to the reported methods. This study separates the drug from its degradants, hence is a typical example of stability indicating assay.

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