International Journal of ChemTech Research
CODEN(USA): IJCRGG ISSN : 0974-4290
Vol. 3, No.2, pp 680-689, April-June 2011

Development of a Validated Stability-Indicating HPLC assay method for Eszopiclone

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Abstract: In the present study, comprehensive stress testing of Eszopiclone was carried out according to ICH guideline Q1A (R2). Eszopiclone is subjected to stress conditions of hydrolysis, oxidation, photolysis and neutral decomposition. Additionally, the solid drug is subjected to 50 °C for 60 days in dry-bath, and to the combined effect of temperature and humidity, with and without light, at 40°C/75% RH. The drug was found to degrade significantly in acidic, alkaline, oxidative, photo and neutral condition. The drug is relatively stable in the solid-state, except formation of minor products under accelerated conditions. Successful separation of drug from degradation products formed under stress conditions is achieved on a Thermo Hypersil BDS–C_{18} (250 mm × 4.6 mm, 5.0 µ) from Germany with isocratic conditions and simple mobile phase containing methanol : water pH adjusted to 2.5 with ortho phosphoric acid (40 : 60) at flow rate of 1 mL/min using UV detection at 304 nm. The method is validated according to ICH guidelines. The developed method is found to be precise, accurate, specific and selective.

Keywords: Eszopiclone, HPLC, Stress studies, Stability indicating method, Validation.

Introduction

The parent drug stability test guideline Q1A (R2) issued by the International Conference on Harmonization (ICH) [1] suggests that stress studies should be carried out on a drug to establish its inherent stability characteristics, leading to identification of degradation products and hence supporting the suitability of the proposed analytical procedures. It also requires that analytical test procedures for stability samples should be stability-indicating and should be fully validated.

Eszopiclone,(*S*)-6-(5-Chloro-2-pyridinyl)-7-oxo-6,7-dihydro-5*H*-pyrrolo[3,4*b*]pyrazin5-yl-4-methyl-1-piperazinecarboxylate (Figure 1), is a nonbenzodiazepine hypnotic agent (viz., a sedative) used as a treatment for insomnia. Eszopiclone is the active dextrorotatory stereoisomer of zopiclone, and belongs to the class of drugs known as cyclopyrrolones. Eszopiclone is a short acting nonbenzodiazepine sedative hypnotic. It has been shown to be safe and effective short term treatment in the elderly and safe in younger adults for 6–12 months [2].

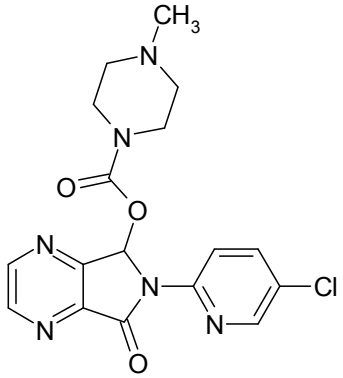

Figure 1 Structure of Eszopiclone

Eszopiclone is a hypnotic agent with a chemical structure unrelated to benzodiazepines, barbiturates, or other drugs with known hypnotic properties, it interacts with the gamma-aminobutyric acid-benzodiazepine ($GABA_BZ$) receptor complex. Subunit modulation of the $GABA_BZ$ receptor chloride channel macromolecular complex is hypothesized to be responsible for some of the pharmacological properties of benzodiazepines, which include sedative, anxiolytic, muscle relaxant, and anticonvulsive effects in animal models. Eszopiclone binds selectively to the brain alpha subunit of the GABA A omega-1 receptor. [3].

Literature review reveals that some analytical methods have been reported for Eszopiclone by UV and difference spectroscopic methods [4], in biological fluids using LCMSMS [5], validated LC method for the estimation of Eszopiclone in bulk and tablet dosage form [6]. Recently stability indicating RP-LC method for determination of Eszopiclone has been reported which is either tedious or expensive method [7]. The LC method uses acetonitrile as the mobile phase which is expensive than methanol as a mobile phase.

In the present study we have proposed new stability indicating simple and reliable RP-LC for determination of Eszopiclone. The aim of the present work is to develop an accurate, specific, repeatable and stability-indicating HPLC method for the determination of Eszopiclone in presence of its degradation products and related impurities for assessment of purity of bulk drug and stability of its bulk dosage forms. The proposed method was validated as per ICH guidelines [8, 9, 10] and its updated international convention .

Experimental

Materials

Wockhardt Pharmaceuticals Ltd. Aurangabad, India, kindly supplied pure drug sample of Eszopiclone as a gift sample of Batch No.: ES71228. It was used without further purification and certified to contain 99.8 % (w/w) on dry weight basis. All chemicals and reagents used were of analytical grade and were purchased from Merck Chemicals, India.

Instrumentation

The LC system consisted of a Pump (model Jasco PU 2080), Intelligent LC pump with sampler programmed at 20 µL capacity per injection was used. The detector consisted of a UV/ VIS (Jasco UV 2075) model operated at a wavelength of 304 nm. Data was integrated using Jasco Borwin version 1.5, LC-Net II/ADC system. The column used was Thermo Hypersil BDS–C_{18} (250 mm × 4.6 mm, 5.0 µ) from Germany.

Forced Degradation Studies

Eszopiclone (100 mg) was weighed accurately and transferred into a 100 mL volumetric flask. Methanol (50 mL) was added and the flask was sonicated for 20 min, and then diluted up to the mark with methanol. An aliquot (2 mL) was further diluted to 100 mL with the same solvent. The final solution contained 20 µg of Eszopiclone per mL of solution. This solution was used for forced degradation to provide an indication of the stability indicating property and specificity of proposed method. In all degradation studies the average peak area of Eszopiclone after application (20 µg/mL) of seven replicates was obtained.

Acid and base induced degradation

Acid decomposition studies were performed by exposing the solution of drug in 1 M hydrochloric acid at 80 °C for 30 min. The studies in alkaline conditions were carried out in 0.1 M sodium hydroxide at 80 °C for 30 min. The resultant solutions were diluted to obtain 20 µg/mL solutions and 20 µL were injected into the system.

Hydrogen peroxide induced degradation

To study hydrogen peroxide induced degradation, the sample was exposed to 3 % hydrogen peroxide at room temperature for a period of 30 min, and then heated in boiling water bath for 10 min to completely remove the excess of hydrogen peroxide. The resultant solutions were diluted to obtain 20 µg/mL solutions and 20 µL were injected into the system.

Photochemical degradation

The photochemical stability of the drug was studied by exposing the stock solution (100 µg/mL) to direct sunlight for 1 h on a wooden plank and kept on terrace. The solution was diluted with methanol to obtain a solution of 20 µg/mL and then 20 µL of the solution was injected into system.

The photochemical stability of the drug was also performed by keeping the stock solution (100 µg/mL) in the stability chamber for 1 h. The solution was diluted with methanol to obtain a solution of 20 µg/mL and then 20 µL of the solution was injected into system.

Neutral Hydrolysis

The degradation behavior of drug in neutral condition was studied by dissolving drug solution in water and solution was refluxed at 80 °C for 5 min.

Optimization of stability indicating HPLC method

The HPLC procedure was optimized with a view to develop stability indicating assay method. Pure

drug along with its degraded products were injected and run in different solvent systems. Initially methanol and water in the ratio of (50 : 50) was tried. It was found that the peak with hump shape was obtained. To improve the peak shape pH of water was adjusted to 2.5 with ortho phosphoric acid. It was found that methanol : water pH adjusted to 2.5 with ortho phosphoric acid (40 : 60) as a mobile phase at flow rate 1 mL/min gave acceptable retention time (t_R), theoretical plates and good resolution of drug and degradation products (Figure 2).

Validation of the method

Validation of optimized LC method was done with respect to following parameters.

Linearity and range

Linearity of the method was studied by injecting six concentrations of the drug prepared in the mobile phase in the range of 4-14 µg/mL in triplicate into the HPLC system keeping the injection volume constant. The peak areas were plotted against the corresponding concentrations to obtain the calibration graphs.

Precision

Precision of the method was verified by repeatability and intermediate precision studies. Repeatability studies were performed by analysis of three different concentrations (4, 8, 12 µg/ mL) of the drug in hexaplicate on the same day. Intermediate precision of the method was checked by repeating studies on three different days. Additionally, the developed HPLC method was checked through separation studies on the mixture of reaction solutions on a different chromatographic system on a different day.

Limit of detection and limit of quantitaiton

In order to estimate the limit of detection (LOD) and limit of quantitation (LOQ), blank methanol was injected six times following the same method as explained above. The signal to noise ratio was determined. LOD was considered as 3:1 and LOQ as 10:1. The LOD and LOQ were experimentally verified by diluting known concentrations of standard solution of Eszopiclone until the average responses were approximately 3 or 10 times the standard deviation of the responses for six replicate determinations.

Robustness of the method

To evaluate robustness of HPLC method, few parameters were deliberately varied. The parameters included variation of flow rate, percentage of methanol in the mobile phase and solvents from different lot were taken. The resolution of drug in a mixture of stressed samples was studied by performing the analyses on a different chromatographic system. Robustness of the method was done at three different concentration levels 4, 8, 12 µg/mL for Eszopiclone. Also robustness was verified by studying the resolution of drug in a mixture of degraded samples on different chromatographic system on a different day.

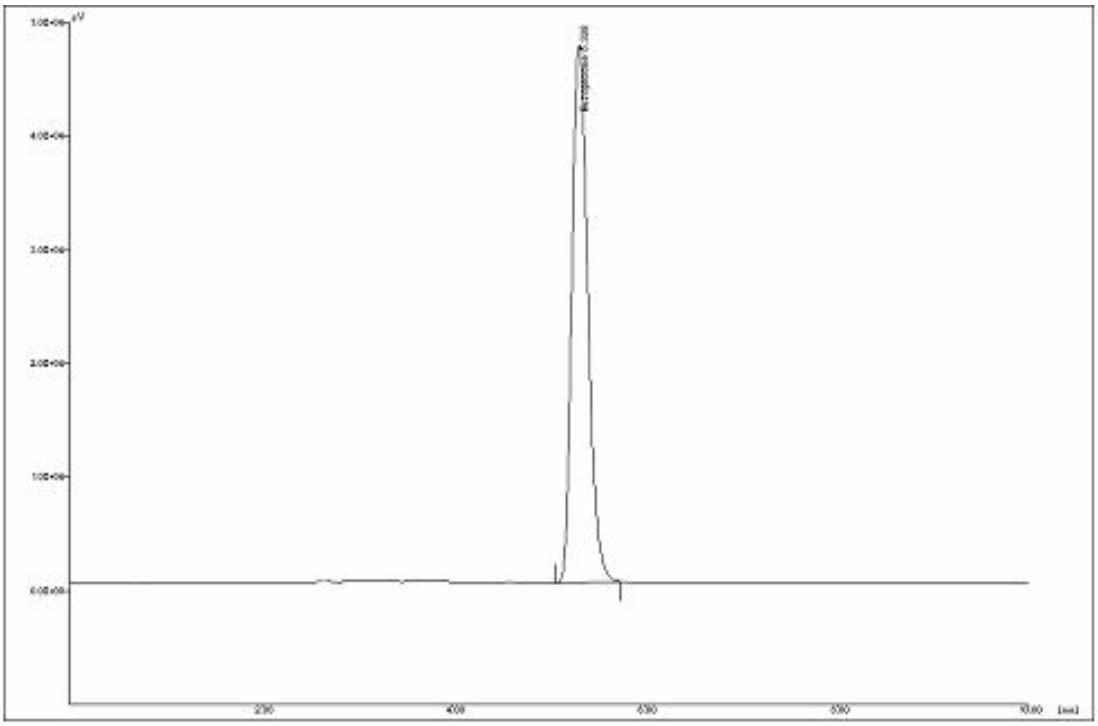

Figure 2 HPLC chromatogram of standard Eszopiclone (20 µg/mL

Specificity

The specificity of the HPLC method was determined by the complete separation of Eszopiclone in presence of its degradation products along with other parameters like retention time, capacity factor, tailing or asymmetrical factor etc.

Accuracy

Accuracy of the developed method was tested by fortifying a mixture of decomposed reaction solutions with three concentrations of drug corresponding to 80, 100 and 120% and determining the recovery of added drug. At each level of the amount six determinations were performed.

Analysis of marketed formulation

To determine the content of Eszopiclone (1 mg) tablets which was procured from local market, twenty tablets were weighed, their mean weight determined and finely powdered. The weight of the tablet triturate equivalent to 1 mg of Eszopiclone was transferred into a 25 mL volumetric flask containing 15-20 mL methanol, sonicated for 30 min and diluted to 25 mL with methanol. The resulting solution was centrifuged at 3000 rpm for 5 min and the drug content of the supernatant was determined (40 µg/mL). Supernatant was taken and after suitable dilution the sample solution was then filtered using 0.45-micron filter (Millipore, Milford, MA). The above stock solution was further diluted to get sample solution 20 µg/mL. A 20 µl volume of sample solution was injected into HPLC, six times, under the conditions described above. The peak areas of the spots were measured at 304 nm and concentrations in the samples were determined using multilevel calibration developed on the same HPLC system under the same conditions using linear regression equation.

Results and discussion

Stability indicating property

HPLC studies of samples obtained on stress testing of Eszopiclone under different conditions using methanol : water pH adjusted to 2.5 with ortho phosphoric acid (40 : 60) as a mobile phase suggested the following degradation behavior.

Hydrolysis

The rate of degradation in acid was slower as compared to that in alkali. Initially 1 M hydrochloric acid was refluxed at 80 °C for 1 h but more than 50 % of the degradation was observed, hence the duration of degradation was decreased. Then, 30 % degradation was observed with 1 M hydrochloric acid at 80 °C for 30 min associated with rise in a major degradation product at retention time 2.95 min (Figure 3).

The drug was found to undergo alkaline degradation faster as compared with that in acid degradation. The reaction in 1 M sodium hydroxide at 80 °C was so fast that around 60 % of the drug was degraded in 30 min. Subsequently, studies were performed by reducing the molarity of sodium hydroxide to 0.1 M. Drug showed degradation of around 15 % in 30 min at 80 °C with rise in a major degradation product at retention time 2.85 min (Figure 4).

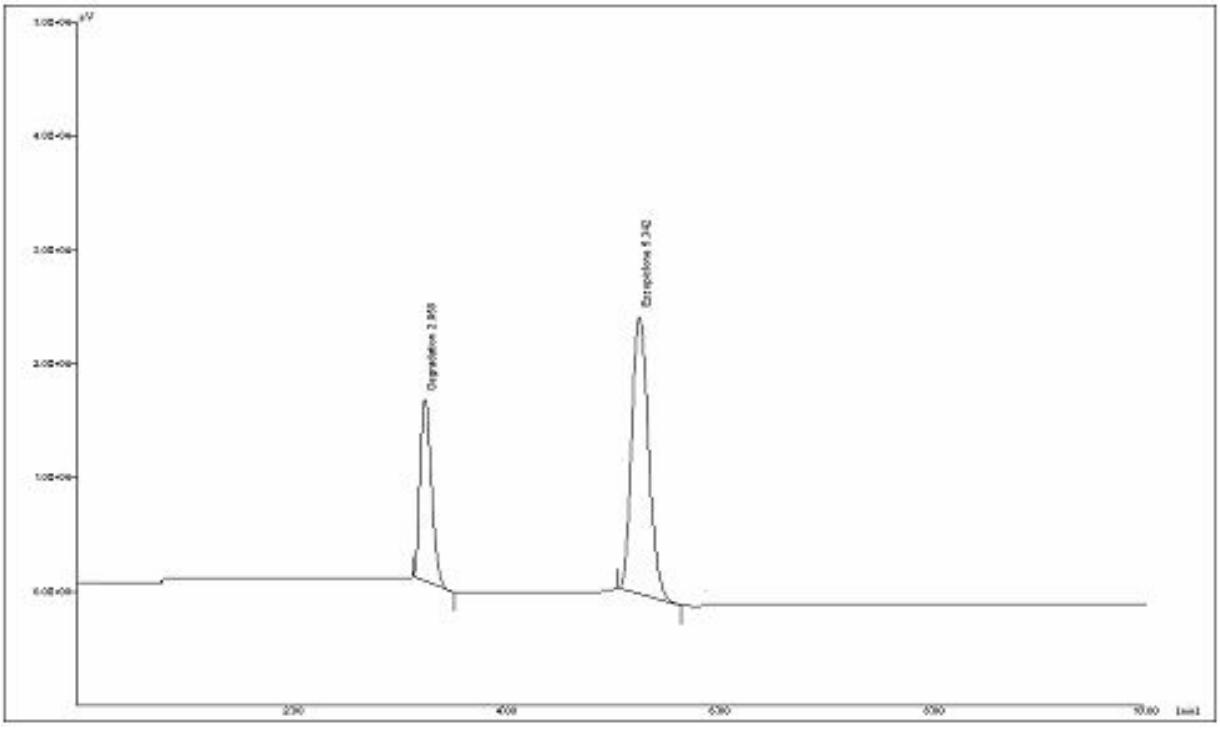

Figure 3 HPLC chromatogram of acid (1 M hydrochloric acid at 80 °C for 30 min).

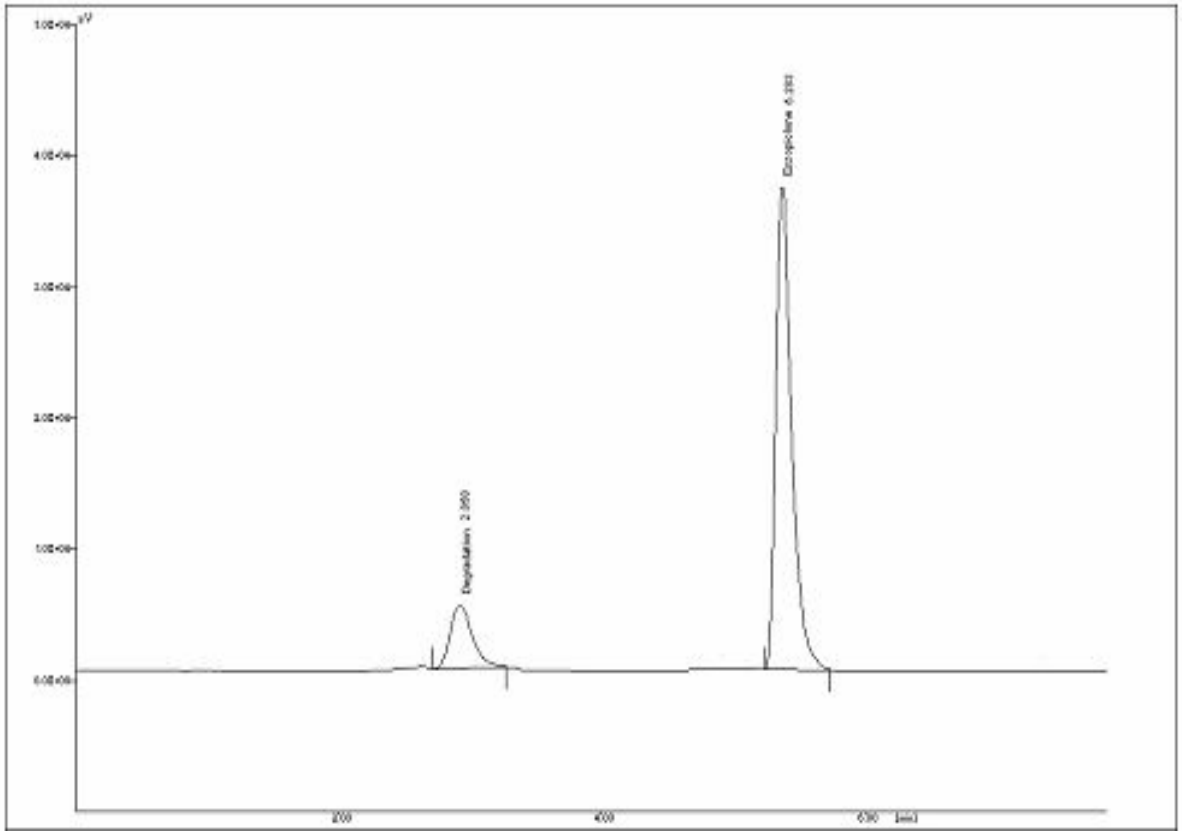

Figure 4 Chromatogram of base (0.1 M sodium hydroxide at 80 °C for 30 min).

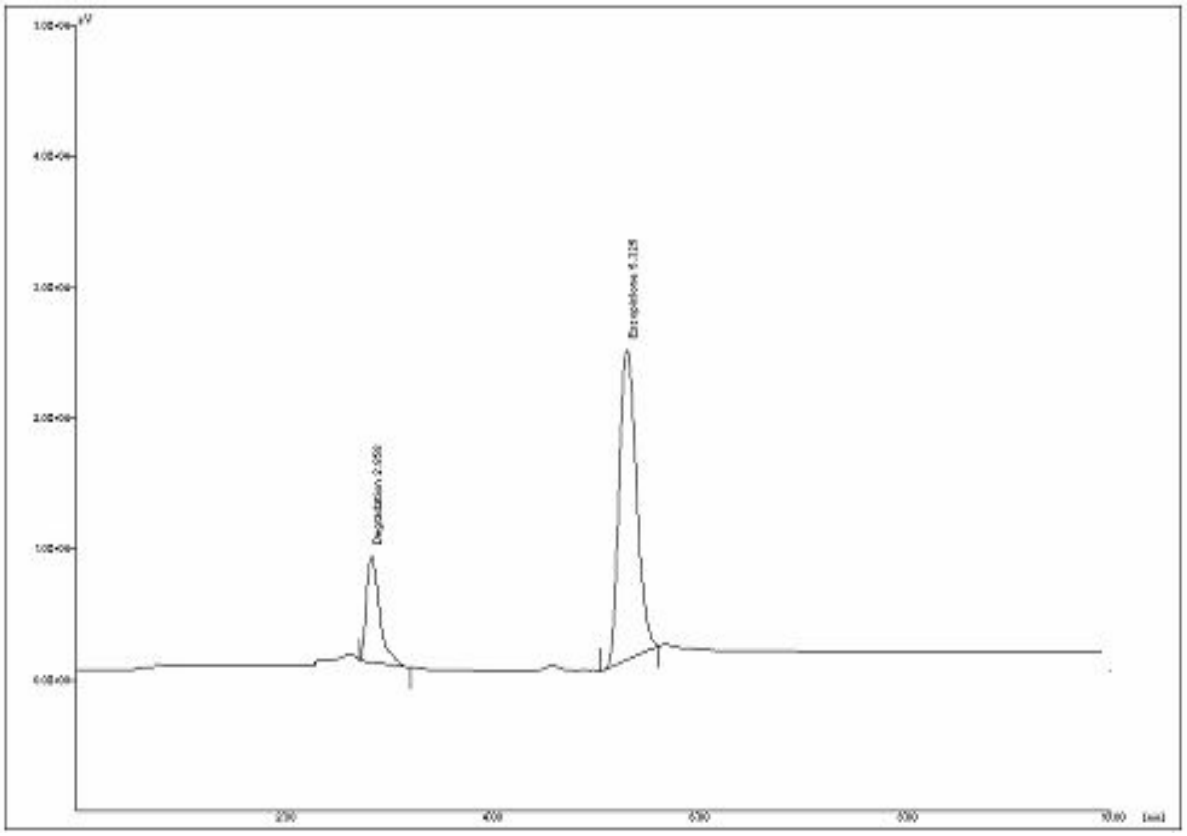

Figure 5 Chromatogram of H_2O_2 (3 % H_2O_2 at room temperature for 30 min).

Oxidation

The drug was found to be highly labile to oxidative degradation. The reaction in 3 % H_2O_2 was carried out at room temperature for 1 h but the degradation was so fast that around 40 % of the drug was degraded, hence the duration of exposure was decreased. Subsequently, studies were performed in 3 % H_2O_2 at room temperature for 30 min. The drug showed a degradation of around 20 % forming major degradation product at 2.85 min (Figure 5).

Photochemical degradation

Eszopiclone was found to be unstable to photochemical degradation as around 15 % of the degradation was seen after exposing drug solution in methanol to sunlight for 1 h forming major degradation products at 2.52 and 2.85 min (Figure 6a).

Around 10-15 % of the degradation was observed after exposing the drug solution in photo stability chamber for 1 h forming major degradation product at 2.85 min (Figure 6b).

Neutral degradation

Upon heating the drug solution in water at 80 °C for 5 min around 15-20 % of the degradation was observed forming major degradation product at 2.85 min (Figure 7).

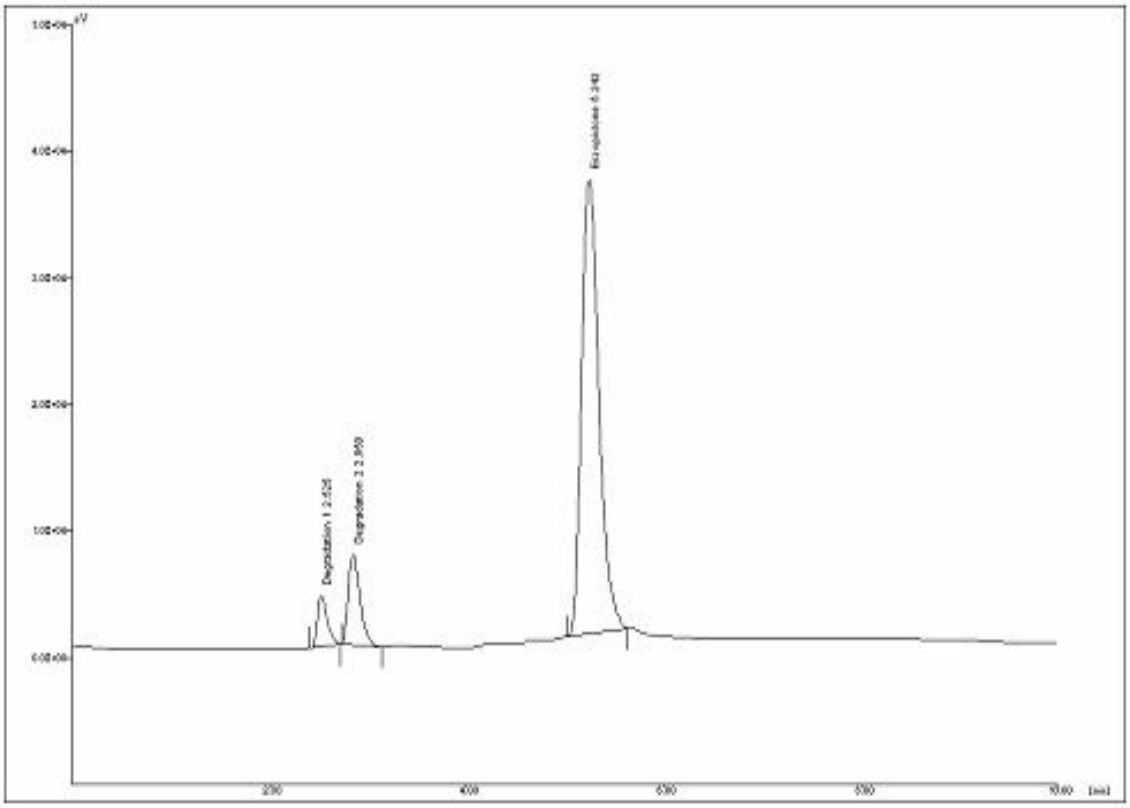

Figure 6a Chromatogram of photochemical degradation (1 h in sunlight).

Table 1-Linearity Studies

Parameters	**Eszopiclone**
Linearity range	4-14 µg/mL
r^2 ± S.D	0.9982 ± 0.76
Slope ± S.D	48670 ± 1025
Interscept ± S.D	39910 ± 9870
Confidence limit of slope[a]	45820 to 51510
Confidence limit of intercept[a]	12510 to 67310
Sy. X	8579

*p<0.0001 : Slope significantly different from zero

[a] 95% confidence limit

Sy.X : Standard deviation of residuals from line

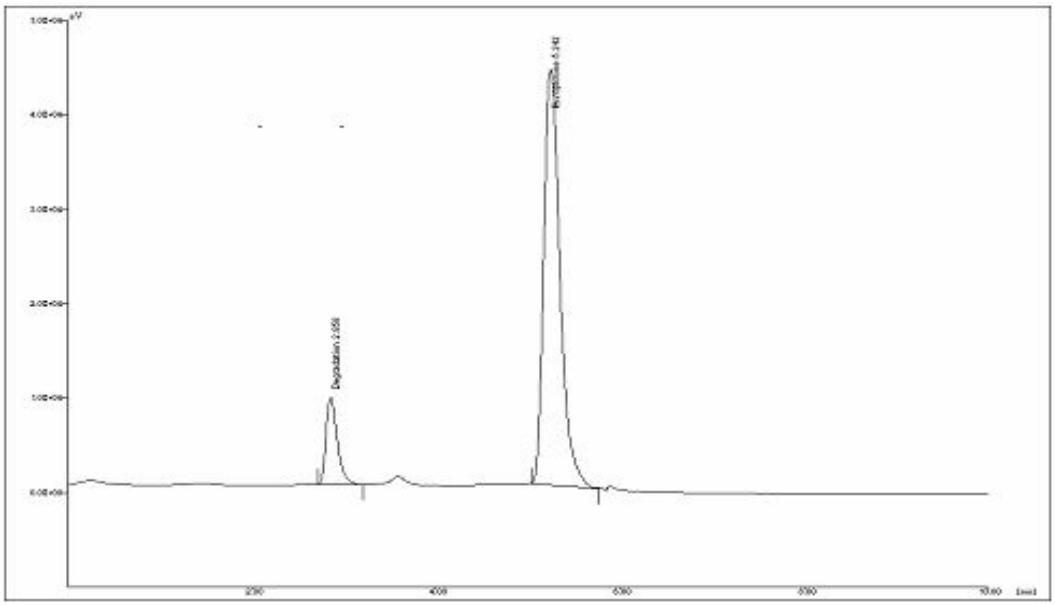

Figure 6b Chromatogram of photochemical degradation (1 h in stability chamber).

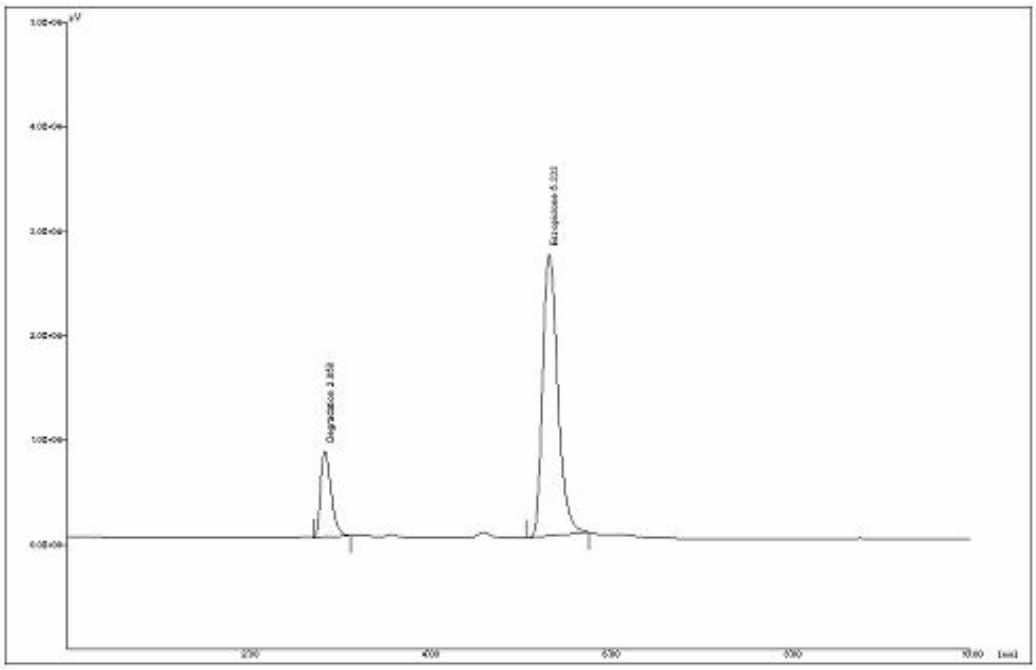

Figure 7 Chromatogram of neutral degradation (water at 80 °C for 5 min).

Validation of the stability indicating method

The results of validation studies on the stability indicating method developed for Eszopiclone in the current study involving methanol : water pH adjusted to 2.5 with ortho phosphoric acid (40 : 60) as a mobile phase are given below.

Linearity

The response for the drug was linear (r^2= 0.9982) in the concentration range between 4-14 µg/mL. The mean (±RSD) values of slope, intercept and correlation coefficient were 48670 (± 1025), 39910 (± 9870) and 0.9982 (± 0.76), respectively Table 1.

Precision

The results of the repeatability and intermediate precision experiments are shown in Table 2. The developed method was found to be precise as the RSD values for repeatability and intermediate precision studies were < 2%, respectively as recommended by ICH guideline. Separation of the drug and different degradation products in stressed samples was found to be similar when analysis was

performed on different chromatographic system on different days.

LOD and LOQ

The signal to noise ratios of 3:1 and 10:1 were considered as LOD and LOQ respectively. The LOD and LOQ were found to be 1 µg/mL and 2 µg/mL respectively.

Robustness of the method

Each factor selected was changed at three levels (-1, 0 and 1). One factor at the time was changed to estimate the effect. Thus, replicate injections (n = 6) of mixed standard solution at three concentration levels were performed under small changes of three chromatographic parameters (factors). Insignificant differences in peak areas and less variability in retention time were observed **(**Table 3). The resolution of drug in the mixture of stressed sample was found to be similar when studies were performed on different chromatographic system on different days indicating that the method has sufficient ruggedness.

Specificity

The specificity of the HPLC method is illustrated in Figure 2, 3, 4, 5, 6a, 6b, 7 where complete separation of Eszopiclone in presence of its degradation products was noticed. The peaks obtained were sharp and have clear baseline separation. The resolution factor for drug from nearest resolving was > 3. The photodiode array scanned all the components present in the mixture in whole wavelength range from 200 to 400 nm and it indicated that there is no degradation peak (hiding) under or unresolved from the analyte peak (pure drug), which also reflected the specificity of the method.

Recovery studies

As shown from the data in Table 4 good recoveries of the drug in the range from 98.80 to 100.47 % were made at various added concentrations, despite the fact that the drug was fortified to a mixture that contained drug as well as degradation product formed at various reaction conditions.

Analysis of marketed formulation

The drug content was found to be 101.07 % ± 1.01. Two different lots of commercially available Eszopiclone tablet were analyzed using the proposed procedures and the results are summarized in Table 5.

Table 2-Precision studies

Concentration (ng/spot)	Repeatability (n=6)			Intermediate precision (n=6)		
	Measured conc. ±SD	(%) RSD	Recovery (%)	Measured conc. ±SD	(%)RSD	Recovery (%)
Eszopiclone						
4	4.03 ± 1.2	0.18	100.75	4.01 ± 1.8	0.14	100.25
8	7.91 ± 2.8	0.46	98.87	7.95 ± 3.3	0.24	99.37
12	11.94 ± 5.1	0.27	99.50	12.01 ± 7.8	0.33	100.08

Table 3 -Robustness testing[a] (n = 3)

Factor[a]	Level	Retention time	Retention factor	Asymmetry
A: Flow rate (mL/min)				
0.9	-1	5.33	1.13	1.31
1.0	0	5.30	1.12	1.28
1.1	+1	5.27	1.10	1.26
Mean ± SD (n = 3)		5.30 ± 0.03	1.11 ± 0.01	1.28 ± 0.02
B: % of methanol in the mobile phase (v/v)				
39	-1	5.31	1.12	1.30
40	0	5.30	1.12	1.28
41	+1	5.29	1.11	1.26
Mean ± SD (n = 3)		5.30 ± 0.01	1.11 ± 0.005	1.28 ± 0.02
C: Solvents of different lots				
First lot		5.30	1.12	1.28
Second lot		5.27	1.10	1.30
Mean ± SD (n = 3)		5.28 ± 0.02	1.11 ± 0.01	1.29 ± 0.01

[a] Three factors were slightly changed at three levels (-1, 0, 1)

Table 4-Recovery studies (n = 6)

Label claim (mg/tablet)	Amount added (mg)	Total amount (mg)	Amount Recovered (mg) ± % RSD	% Recovery
1	1 (80%)	1.8	1.78 ± 0.66	98.88
1	1 (100%)	2.0	2.01 ± 1.01	100.50
1	1 (120%)	2.2	2.16 ± 0.85	99.09

Table 5-Analysis of commercial formulation

Eszopiclone (1 mg)	Eszopiclone found (mg per tablet)	
	Mean ± SD (n= 6)	Recovery (%)
1st Lot	0.99 ± 0.57	99.03
2nd Lot	0.98 ± 0.44	98.87

Conclusion

HPLC method was developed and validated as per ICH guidelines for stability-indicating studies of Eszopiclone. UV detection allowed an accurate quantitation of chromophoric compounds.

In this study, intrinsic stability of Eszopiclone was established using various ICH recommended stress conditions. The drug as such was very stable in solid form but was found to be unstable in methanolic solution. In the latter case, unknown decomposition products were formed under stress conditions. The drug was found to degrade significantly in acidic, alkaline oxidative, photo and neutral condition.

The drug was analysed in the presence of different degradation products by HPLC method using Thermo Hypersil BDS–C_{18} (250 mm × 4.6 mm, 5.0 µ) from Germany with isocratic conditions and simple mobile phase containing using methanol : water pH adjusted to 2.5 with ortho phosphoric acid (40 : 60) at flow rate of 1 mL/min using UV detection at 304 nm. The procedure has been evaluated for the linearity, accuracy, precision and robustness in order to ascertain the suitability of the analytical method. The method was also applied to marketed samples. It has been proved that the method is selective and linear between concentration range 4-14 µg/mL. LOD was found to be 1 µg/mL and LOQ was found to be 2 µg/mL.

Acknowledgements

The authors would like to thank, Wockhardt Ltd. (Aurangabad, India) for providing a gift sample of standard Eszopiclone. The authors would like to thank, Dr. K. R. Mahadik, Principal, Poona College of Pharmacy, Pune, India for providing necessary facilities to carry out the work. The authors would also like to thank AICTE for providing financial support for carrying research work.

References

1. ICH (2003) Stability Testing of New Drug Substances and Products, International Conference on Harmonization, Q1A (R2), IFPMA, Geneva, Switzerland.
2. Eszopiclone - Wikipedia, the free encyclopedia.
3. Eszopiclone (DB00402) – Drug bank.
4. Anandakuma K., Kumaraswamy G., Ayyappan T., Sankar A.S.K. and Nagavalli D., Estimation of Eszopiclone in Bulk and in Formulation by Simple UV and Difference Spectroscopic Methods, Research Journal of Pharmacy and Technology, 2010, 3,202.
5. Meng M., Rohde L., Capka V. and Bennett P., Development and Validation for the Quantitation of Eszopiclone in Human Plasma Using LC/MS/MS. Tandem Labs., Chiral Chromatographic Method, 2007, 1-14.
6. Anandakuma K., Kumaraswamy G., Ayyappan T., Sankar A.S.K. and Nagavalli D., Validated RP-HPLC Method for the Estimation of Eszopiclone in Bulk and Tablet Dosage Form, Asian Journal of Research in Chemistry, 2010, 3,63.
7. Kumar R.N., Rao G.N. and Naidu P.Y. Stability Indicating RP-LC Method for Determination of Eszopiclone in Bulk and Pharmaceutical Dosage

Forms, Asian journal of research in chemistry, 2010, 3,374.

8. ICH, Q1A Stability Testing of New Drug Substances and Products, International Conference on Harmonization, Geneva October 1993.
9. ICH, Q2A Validation of Analytical Procedure: Methodology International Conference on Harmonization, Geneva October 1994.
10. ICH, Q2B Validation of Analytical Procedure: Methodology International Conference on Harmonization, Geneva March 1996.

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