ISSN : 0974-7419 Volume 12 Issues 6

Trade Science Inc.

Analytical CHEMISTRY

An Indian Journal

Full Paper

ACAIJ, 12(6) 2013 [205-210]

A stability-indicating RP-HPLC determination of eszopiclone in drug substance

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ABSTRACT

A simple, specific, accurate and stability-indicating reversed phase high performance liquid chromatographic method was developed for the determination of Eszopiclone in drug substance, using Inertsil ODS-3 column (250 mm Length x 4.6 mm ID, 5 µ Particle Size) and isocratic mobile phase composed of buffer and acetonitrile in the 62:38 ratio, at a flow rate of 1.5 mL min^{-1} with UV detection at 303 nm. The Retention time of Impurity B, Impurity C, Impurity A and Eszopiclone were found to be 3.867 min, 7.305 min, 18.729 min and 30.817 min respectively. The proposed method was validated as per ICH guidelines[1-2]. The Linearity for Eszopiclone was in the range of 50-150 µg mL^{-1}. The recovery was found to be in the range of 100.4-101.3%. The detection limit and quantification limit were found to be in the range of 0.1 µg mL^{-1} and 0.25 µg mL^{-1} respectively. Eszopiclone was subjected to acid, alkali and neutral hydrolysis, chemical oxidation and dry environmental condition. The degraded product peaks were well resolved from the pure drug peak with significant difference in their retention time values. This proposed method was validated and successfully employed for quantitative analysis of Eszopiclone in drug substance.

KEYWORDS

ICH Guidelines;
Validation;
Column liquid chromatography;
Drug substance;
Eszopiclone.

INTRODUCTION

Eszopiclone (*S*)-6-(5-Chloro-2-pyridinyl)- 7-oxo-6,7-dihydro- 5*H*-pyrrolo 3,4-*b*]pyrazin-5-yl-4-methyl-1-piperazinecarboxylate. ($C_{17}H_{17}ClN_6O_3$). Figure 1 is a non-benzodiazepine hypnotic used for treatment of insomnia. Eszopiclone is the active dextro-rotatory stereoisomer of zopiclone, and belongs to the class of drugs known as cyclopyrrolones. The literature survey reveals method for determination of Eszopiclone in drug product by HPLC but no method is reported for Eszopiclone in drug substance using its probable impurities[3-7]. A new stability indicating RP-HPLC method was thus developed for the determination of Eszopiclone from drug substance in presence of its degradation products. The method described is specific, precise and accurate for determination of Eszopiclone in drug substance.

Chemicals and reagents

Standard and Sample were arranged from Bijbeep Enterprises. Sodium lauryl sulphate, Sodium dihydrogen orthophosphate, Acetonitrile and orthophosphoric acid were from Rankem. Double distilled water was employed throughout the work. All dilutions were performed in standard volumetric flasks.

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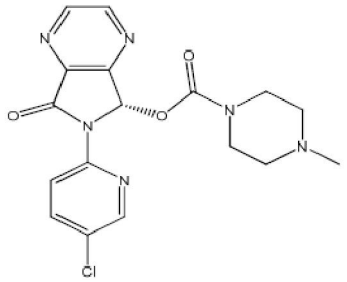

Figure 1 : Structure of eszopiclone.

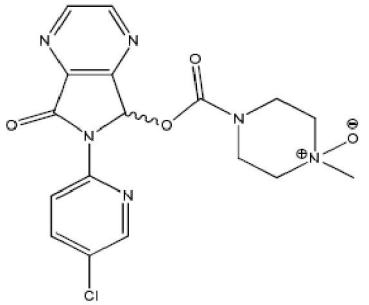

Figure 2 : Structure of impurity A.

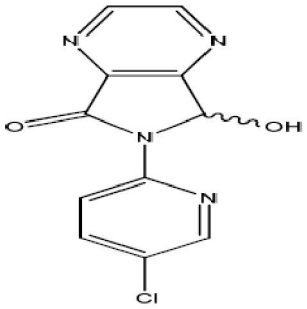

Figure 3 : Structure of impurity B.

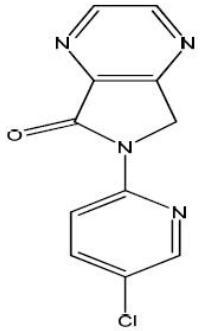

Figure 4 : Structure of impurity C.

EXPERIMENTAL

Method development and optimization of chromatographic conditions

The aim was to develop a suitable stability indicating RP-LC method for the analysis of Eszopiclone in presence of its degradation products, accordingly different mobile phases were tried. The criteria employed for selecting the mobile phase for the analysis of the drug were better separation of drug along with its probable impurities, time required for the analysis and the cost involved. Chromatographic separation was performed with Agilent 1100 series High performance liquid chromatography having quaternary gradient pump, equipped with auto sampler and a photo-diode array detector. The UV spectra of Eszopiclone and its impurities were scanned on photo diode array detector for selecting the working wavelength. Peak purity of Eszopiclone and its impurities were checked using photo-diode array detector. Chromatograms and data were recorded by means of chemstation software. Inertsil ODS-3 (250 mm x 4.6 mm, 5 µ) was used for the analysis. The mobile phase comprising of buffer and acetonitrile in the 62:38 ratio was used. The mobile phase is used as diluent for preparation of standard and sample solutions. The column temperature was maintained at 30°C. The system was run at a flow rate of 1.5 mL min^{-1} and effluent was monitored at 303 nm for determination of Eszopiclone.

Sample was injected using a 20 µL fixed loop, and the total run time was 60 min

Preparation of mobile phase and diluent

The buffer solution was prepared by dissolving 8.1 gms of sodium lauryl sulfate and 1.6 gms of sodium dihydrogen orthophosphate in 1000 mL of water. The pH of the buffer solution was adjusted to 4.0 with the help of orthophosphoric acid. The 620 mL buffer solution was mixed with 380 mL of acetonitrile. The solution was sonicated for 10 minutes and used as mobile phase and also as diluent.

Resolution mixture solution

Resolution mixture solution was prepared by dissolving 1.5 mg of each Impurity A, Impurity B, Impurity C and 100 mg of Eszopiclone using diluent into 100 mL standard volumetric flask.

Standard solution

Stock solution was prepared by weighing 24.92 mg of analytically pure Eszopiclone (99.5 %) and transferring into 25 mL standard volumetric flask. Volume was made upto the mark with the diluent, which gave

1000 μg mL^{-1} of the drug. The solution was further diluted with the same diluent to obtain final concentration of 100 μg mL^{-1}

Preparation of sample solution

The sample solution was prepared by dissolving about 25.0 mg of sample in diluent in a 25 mL standard volumetric flask. 5 mL of this solution was transferred to 50 mL volumetric flask and diluted upto the mark with diluent.

Linearity stock solution

The Linearity stock solution of Eszopiclone (1000 μg mL^{-1}) was prepared by dissolving 24.92 mg of Eszopiclone (99.5 %) in diluent in a 25 mL standard volumetric flask.

RESULTS AND DISCUSSION

Method development

Several mobile phases using different organic solvents as part of mobile phase were tried. Phosphate buffer and acetonitrile in the ratio of 50:50 v/v was chosen for initial trial with a C_{18} column (25 cm, 4.6 mm and 5 μ). Flow rate was 1.5 mL min^{-1}. When system suitability solution was injected the, impurity B and Impurity C did not resolve effectively also peak shape of eszopiclone was not symmetrical.

To improve the peak shape and retention of Eszopiclone, Sodium lauryl sulfate was used in buffer with sodium dihydrogen orthophosphate and pH adjusted to 4.0 using orthophosphoric acid. The mobile phase comprising of buffer and acetonitrile in the ratio 62:38 v/v was used. When system suitability solution was injected in the above conditions the resolution between Impurity B and Impurity C was found greater than 2.0 also peak shape of eszopiclone was found symmetrical and the typical retention times of Impurity B, Impurity C, Impurity A and Eszopiclone were 3.867 min, 7.305 min, 18.729 min and 30.817 min respectively.

System suitability

System suitability tests are used to verify that the reproducibility of the equipment is adequate for the analysis to be carried out. System suitability tests were performed as per the general chapter <621> in USP 35 NF 30 to confirm the suitability and reproducibility of the system. The test was carried out by injecting 20 μL resolution mixture solution and standard solution of Eszopiclone. In resolution mixture solution, the resolution between all impurities and Eszopiclone was found to be more than 2.0. Six replicate injections of standard solution were made. The % The relative standard deviation (RSD) value of Eszopiclone was 0.09. The %RSD value was found to be satisfactory and meeting the requirements of the general chapter <621> in USP 35 NF 30 (%RSD not more than 2.0). Number of theoretical plates, resolution, tailing factor were determined and are presented in TABLE 1.

TABLE 1 : Result of system suitability.

Component	Retention Time	Resolution	Tailing Factor	Theoretical Plates
Impurity B	3.867 min	----	1.04	3518
Impurity C	7.305 min	5.06	1.01	5333
Impurity A	18.729 min	14.05	1.11	4009
Eszopiclone	30.817 min	10.11	1.02	9899

Method validation

Method validation was done as per ICH guidelines[1,2].

Specificity (Forced degradation study)

Forced degradation study is carried out to know in advance likely degradation products that may be generated during stability study or shelf life. In forced degradation study, it is observed that under dry environmental conditions, i. e. UV radiation and thermal exposure, no major degradation of sample was observed. In aqueous condition sample shows no significant degradation. Alkaline condition causes significant degradation of sample with assay value of Eszopiclone reduced to 80.7%. Instantaneous degradation of 4.83% was observed in the sample when treated with 2.0 mL of 0.1N NaOH kept at room temperature. The mass balance was found 87.1%. In acidic condition sample showed 1.79% degradation with assay value of Eszopiclone reduced to 96.9%. Eszopiclone showed 7.83% of degradation with assay value of 80.6% in oxidation condition when treated with 3% Hydrogen peroxide. The degradant impurity at Relative Retention Time (RRT) 0.23 min showed maxima at about 279 nm

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and impurity at RRT 0.05 min showed maxima at 240 nm. In all above conditions, where the degradation is observed, main peak of Eszopiclone is found to be pure and no other peak is merged in it (as indicated by PDA detector). All degradants are well separated from Eszopiclone peak indicating specificity and stability indicating nature of the method. Refer figure 5 to 13 for chromatograms of forced degradation study.

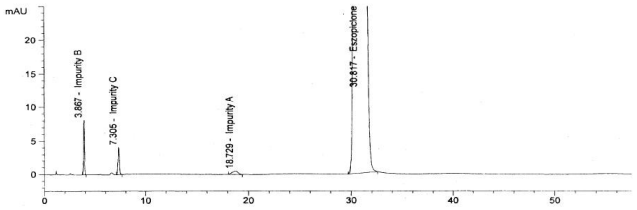

Figure 5 : Chromatogram of resolution mixture solution.

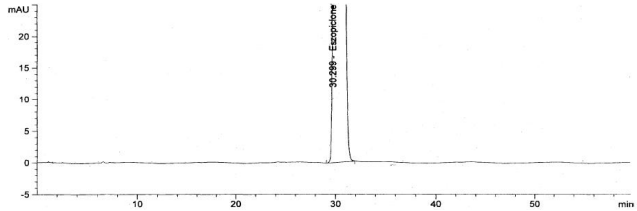

Figure 6 : Chromatogram of standard solution.

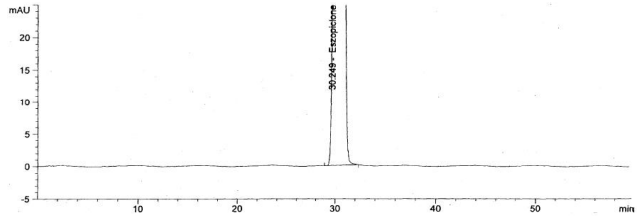

Figure 7 : Chromatogram of sample solution.

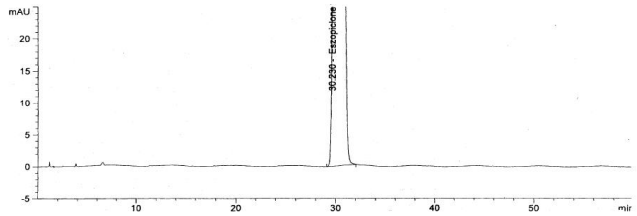

Figure 8 : Chromatogram of sample exposed to UV condition.

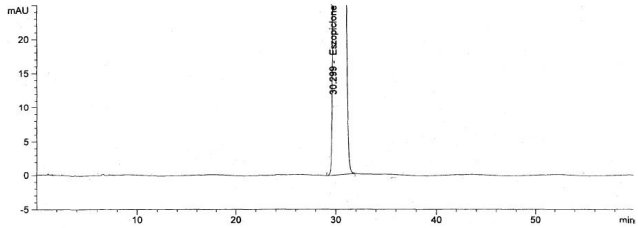

Figure 9 : Chromatogram of sample exposed to heat.

Linearity

Linearity was evaluated by analysis of working standard solution of Eszopiclone at seven different concentrations. The range of linearity was from 50 – 150 µg mL^{-1}. The peak area and concentration of Eszopiclone was subjected to regression analysis to calculate the calibration equations and correlation coefficient. The regression data obtained for the Eszopiclone is represented in TABLE 2. The result shows that within the concentration range mentioned above, there was an excellent correla-

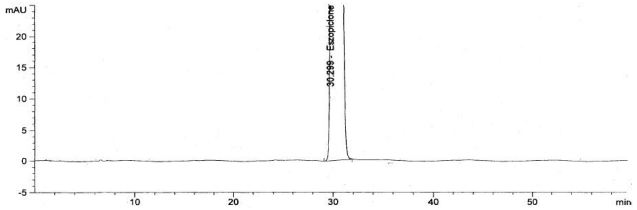

Figure 10 : Chromatogram of sample exposed to aqueous condition.

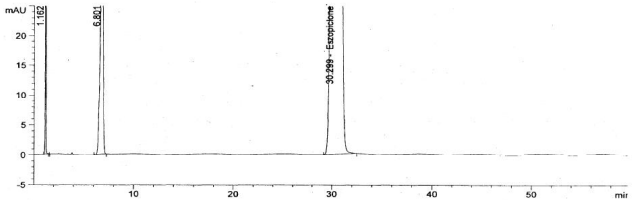

Figure 11 : Chromatogram of sample exposed to acidic condition.

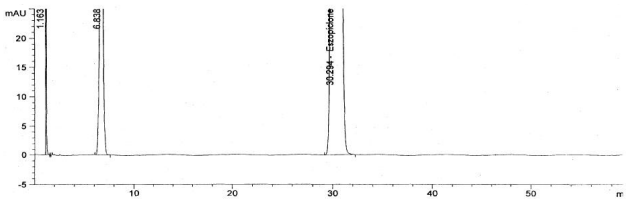

Figure 12 : Chromatogram of sample exposed to basic condition.

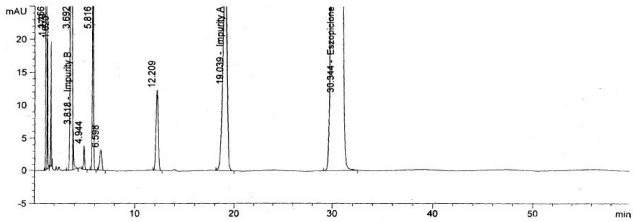

Figure 13 : Chromatogram of sample exposed to oxidation condition.

tion between peak area and concentration.

Sensitivity

Sensitivity was determined by establishing the limit of detection (LOD) and limit of quantification (LOQ). The detection limit of an individual analytical procedure is the lowest concentration of an analyte which can be detected but not necessarily quantified as an exact value. The quantitation limit of an individual analytical procedure is the lowest amount of analyte which can be quantitatively determined with suitable precision and accuracy. LOD was the concentration that yielded signal to noise ratio (S/N) 3:1 and LOQ was the concentration that yielded signal to noise ratio (S/N) 10:1. The LOD and LOQ of Eszopiclone was experimentally determined by six replicate injections. The LOD of Eszopiclone was found to be 0.1µg mL^{-1}. The LOQ of Eszopiclone was found to be 0.25µg mL^{-1}.

TABLE 2 : Results of linearity experiment.

Analyte	Slope	Intercept	Correlation Coefficient
Eszopiclone	30.953	-46.506	0.999

Precision

Repeatability was studied by carrying out system precision. System precision was determined from results for six replicate injections of the standard solution. The relative standard deviation (RSD) was less than 2.0%. Method precision was determined from results of six independent determinations at 100% of the test concentrations of Eszopiclone. The % RSD for Eszopiclone was found to be 0.12.

TABLE 3 : Results of precision experiment.

Parameter	Mean Assay (%)	Standard Deviation	RSD (%)
Method Precision	100.4	0.12	0.12

Accuracy

To study accuracy of the method, recovery experiment was carried out by applying the standard addition method. A known quantity of drug substance corresponding to 80%, 100% and 120% of the working concentration of Eszopiclone was added. Each set of addition was repeated three times. The accuracy was expressed as the percentage of analyte recovered by the assay. TABLE 4 lists the recoveries of the drug from a series of spiked concentrations. The results indicate the method is highly accurate for determination of Eszopiclone.

Robustness

Robustness of the method was studied by deliberate change in experimental condition and evaluating resolution between Impurity A and Eszopiclone. The effect of flow rate on system suitability parameters was studied by changing 0.2 units of flow rates i.e. 1.3 and 1.7 mL min^{-1}. The effect of change in column temperature was studied at 25°C and 35°C. The effect of change in pH of buffer was studied at 3.8 and 4.2. In all the above varied conditions, the components of the mobile phase were held constant. The resolution between Impurity A and Eszopiclone was found to be greater than 2.0.

Solution stability

The solution stability of Eszopiclone was carried out by leaving the test solutions of sample in a tightly capped volumetric flask at room temperature for 48

TABLE 4 : Results of accuracy experiment.

Recovery Levels	Amount of Eszopiclone in sample solution (ppm)	Amount of Eszopiclone Standard spiked (ppm)	Total amount Eszopiclone in accuracy solution (ppm)	Eszopiclone recovered (ppm)	% Recovery
80% Level	49.45	30.25	79.70	80.32	100.8
	49.45	30.31	79.76	80.55	101.0
	49.45	30.09	79.54	80.61	101.3
100% Level	49.45	50.29	99.74	100.40	100.7
	49.45	50.41	99.86	100.22	100.4
	49.45	50.56	100.01	100.56	100.5
120% Level	49.45	70.06	119.51	120.75	101.0
	49.45	70.40	119.85	120.55	100.6
	49.45	70.29	119.74	120.43	100.6

hrs. After 12 hrs, 24 hrs and 48 hrs the same sample solution was assayed against freshly prepared standard solution. The % RSD of assay of Eszopiclone during solution stability was within 1.0. No significant changes were observed in the content of Eszopiclone during solution stability experiment. Sample solutions used during the experiment were stable upto the study period of 48 hrs.

CONCLUSION

The stability indicating RP-LC method developed for quantitative determination of Eszopiclone in drug substance is specific, precise, accurate and robust. The method was validated showing satisfactory data for all the method validation parameters tested. The developed method is stability indicating and can be used for routine analysis of production samples and also to check the stability study of Eszopiclone in raw material.

REFERENCES

[1] ICH stability testing of new drug substances and products, International conference on harmonization, Q1A (R2) **(2003)**.

[2] ICH, Q2B Validation of analytical procedures, International conference on harmonization.

[3] Sunil R.Dhaneshwar, Vidhya K.Bhusari; Development of a validated stability-indicating HPLC assay method for eszopiclone, International Journal of Chem.Tech.Research, **3(2)**, 680-689 **(2011)**.

[4] K.Anandakuma, G.Kumaraswamy, T.Ayyappan, A.S.K.Sankar, D.Nagavalli; Estimation of eszopiclone in bulk and in formulation by simple UV and difference spectroscopic methods, Research Journal of Pharmacy and Technology, **3**, 202 **(2010)**.

[5] M.Meng, L.Rohde, V.Capka, P.Bennett; Development and validation for the quantitation of eszopiclone in human plasma using LC/MS/MS. tandem labs. Chiral chromatographic method, 1-14 **(2007)**.

[6] K.Anandakuma, G.Kumaraswamy, T.Ayyappan, A.S.K.Sankar, D.Nagavalli; Validated RP-HPLC method for the estimation of eszopiclone in bulk and tablet dosage form, Asian Journal of Research in Chemistry, **3**, 63 **(2010)**.

[7] R.N.Kumar, G.N.Rao, P.Y.Naidu; Stability indicating RP-LC method for determination of eszopiclone in bulk and pharmaceutical dosage, Int.J.Chem. Tech.Res., **3(2)**, 689 **(2011)**.