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Stability Indicating Analytical Method Validation For determination of Related Substances of Eszopiclonein Tablet Dosage Form

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Abstract: Eszopiclone is a non benzodiazepine hypnotic used as a treatment for insomnia. Eszopiclone isthe active dextrorotatory stereoisomer of zopiclone, and belongs to the class of drugs known as cyclopyrrolones. A simple sensitive, stability-indicating reversed-phase high-performance liquid chromatographic method was developed for the determination of eszopiclone and related impurities in tablet dosage form. The chromatographic separation was achieved on an Zorbax XDB C18 column(250 3 4.6 mm, 5 mm), using a mobile phase consisting of 0.05M monobasic sodium phosphate buffer containing 0.7% sodium laurylsulfate (pH 3.5) and acetonitrile in the ratio of 60:40 (v/v), at a flow rate of 1.5 mL/min and temperature of 40°C. Quantification was achieved with photodiode array detection at 303 nm. The described method showed excellent linearity over a range of limits of quantification to 4.8 mg/mL (150% of specification limit; i.e., 3.2 mg/mL). The drug product was subjected to the stress conditions of oxidative, acid, base, thermal and photolytic degradation. Eszopiclone degradation was observed in acid hydrolysis, base hydrolysis and peroxide stress conditions. Eszopiclone was stable in thermal and photolytic degradation conditions. The method is validated for the quantification of impurities and degradation products of eszopiclone in tablet dosage form.
Keywords: Eszopiclone (ESZ), Hypnotic agent, Analytical Method, Validation, High performance Liquid Chromatography.

Introduction

Eszopiclone is the active dextrorotatory stereoisomer of zopiclone, and belongs to the class of drugs known as Cyclopyrrolones. Eszopiclone is a short acting non-benzodiazepine 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. Eszopiclone is a white to light-yellow crystalline solid, very slightly soluble in water, slightly soluble in ethanol, and soluble in phosphate buffer (pH 3.2)[1-3].

Eszopiclone (ESZ)is a non benzodiazepine hypnotic agent. Hypnotic (also called soporific) drugs are a class of psychoactive, whose primary function is to induce sleep and to be used in the treatment of insomnia and in surgical anesthesia. When used in anesthesia to produce and maintain unconsciousness, Hypnotic drugs are regularly prescribed for insomnia and other sleep disorder [4-5].ESZ is Calcium channel blocker used in the treatment of insomnia and belongs to the class of drugs known as Cyclopyrrolones. Eszopiclone acts on benzodiazepine binding site situated on $GABA_A$ neurons as an agonist. Cytochrome P450 (CYP) isozymes CYP3A4 and CYP2E1 are involved in the biotransformation of ESZ, thus, drugs that induce or inhibit these CYP isozymes may affect the metabolism of ESZ [6]. The objective of this research work was to develop a stability-indicating isocratic LC method for the determination of eszopiclone and its degradation impurities in tablet dosage form. Forced degradation studies were performed on the drug product to show the stability-indicating capability of the method. All of these studies were performed in accordance with established

InternationalConference on Harmonization (ICH) guidelines [7]. The developed method is stability-indicating, simple, robust, precise and accurate for the determination of eszopiclone and degradation impurities in tablet dosage form. The method is capable of separating the peaks due to the degradation products from the main peak. The method was validated as per ICH requirements [8-9], and thus is useful for routine analysis in quality control laboratories.

Experimental

Materials and reagents

All standards and eszopiclone tablets (Brand name: Zopipure (2mg) Manufacturer Emcure Pharmaceuticals Ltd,Type tablets) were purchased from the Indian market. High-performance liquid chromatography (HPLC) grade acetonitrile, and analytical grade sodium lauryl sulphate, sodium dihydrogen phosphate and orthophosphoric acid, were purchased from Merck (Darmstadt, Germany). Water used was obtained by using a Millipore MilliQ Plus water purification system (Billerica, MA).

Table No.1: List of Instrument Used

Sr No	Instrument	Make	Software	Detector/Model No
1	HPLC	Waters	Empower Software	2489 dual wavelength
2	HPLC	Waters	Empower Software	2998 PDA Detector
3	Sonicator	Lab India	NA	NA
4	Weight balance	Mettler Toledo	NA	ML204
5	Oven	Thermo lab	NA	GMP
6	Photolytic Chamber	Thermo lab	NA	GMP

HPLC Method

The chromatographic system was a Waters 2998 HPLC equipped with photodiode array (PDA) detector comprised of a degasser, a quaternary pump, an auto injector, a column compartment. Chromatographic separation was achieved in isocratic mode. An Zorbax XDB C18 column, 250*4.6 mm,* 5 mm, was used for isocratic separation. A mobile phase consisting of 0.05M monobasic sodium phosphate buffer containing 0.6% sodium lauryl sulfate (pH 3.5) and acetonitrile in the ratio of 60:40 (v/v), at a flow rate of 1.5 mL/min and temperature of 40°C. Quantification was achieved with PDA detection at 302 nm. A typical chromatogram of blank solution is shown in Figure 1 and a typical chromatogram of the control sample and spiked solution of eszopiclone and impurities is shown in Figure 2 & figure 3.

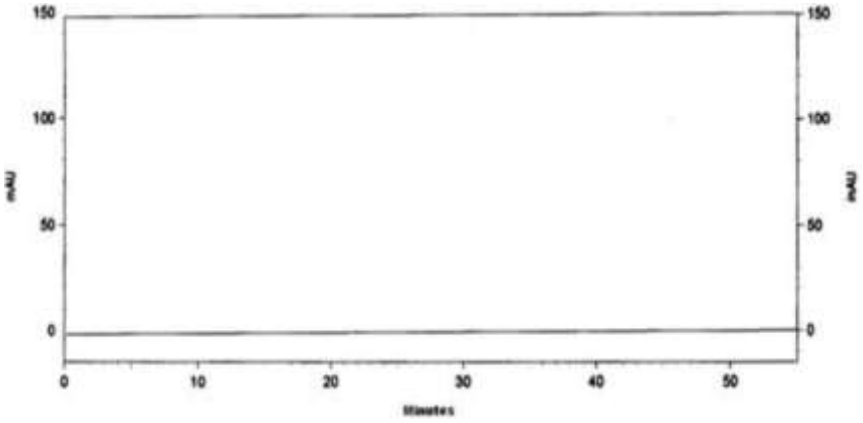

Figure 1. Typical chromatogram of blank solution.

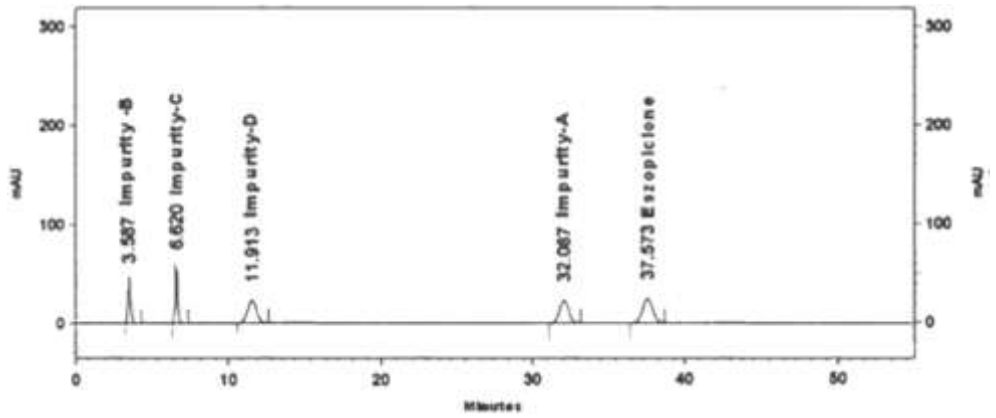

Figure 2. Typical chromatogram of spiked solution of eszopiclone and impurities.

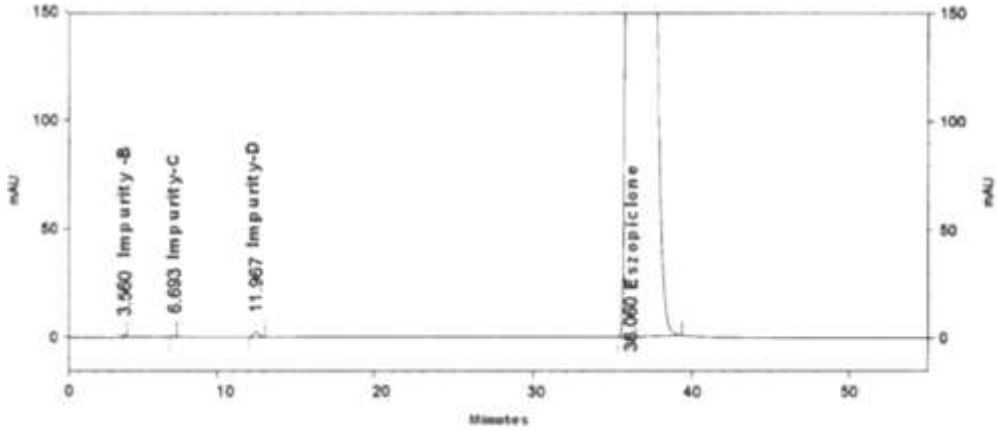

Figure 3. Typical chromatogram of Control sample solution.

Analytical parameters and validation

The optimized chromatographic conditions were validated by evaluating specificity, linearity, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ), robustness and system suitability, in accordance with ICH Q2 (R1) guidelines.

Result and Discussion:

Specificity:

The specificity of a method is its suitability for the analysis of as ubstance in the presence of potential impurities. Stress testing of a drug substance can help to identify likely degradation products, which can establish degradation pathways and the intrinsic stability of the molecule. The peak purity of the eszopiclone was found to be satisfactory under different stress conditions. The specificity of the LC method for eszopiclone was determined in the presence of their known impurities.

Forced Degradation Studies:

The stress degradation studies performed on the drug product included acid hydrolysis (5 mL of 5N HCl for 0 Hrs and 24 Hrs), base hydrolysis (5 mL of 2N NaOH for 0 Hrs and 24 Hrs), oxidation(5 mL of 50% H2O2 for 0 Hrs and 24 Hrs), thermal (105°C for 72 h),humidity (25°C, 75% RH for seven days) and photolytic degradation(drug product exposed to UV light for 1.2million lux/hrs). The stress studies were performed as per ICH recommendations. Peak purity was checked for the eszopiclone peak by using a PDA detector in stress samples. No peaks were found at the retention times of eszopiclone and its known impurities in blank and placebo chromatograms, which proved no interference from blank and placebo. Slight degradation was observed when the drug product was subjected to peroxide stress conditions and stability was observed in

photolytic and humidity stress conditions. Eszopiclone was sensitive for acid stress conditions and significantly degraded into an unknown impurity. Peak purity results from the PDA detector for the peaks produced by the degradation of eszopiclone confirmed that all peaks were homogeneous and pure for all analysed stress samples (Table no.2).

Table No.2: Stress Testing (Forced Degradation) Data

Stress Condition	Eszopiclone net degradation	Purity Angle	Purity Threshold
Control Sample	NA	0.425	0.982
Acid Sample 0 Hrs	31.2	0.456	0.769
Base Sample 0 Hrs	6.2	0.685	0.882
Base Sample 24 Hrs	17.2	0.982	1.102
Peroxide Sample 0 Hrs	5.1	0.652	1.212
Peroxide Sample 24 Hrs	11.2	0.743	1.252
Photo Sample	1.8	1.124	2.143
Humidity Sample	Stable	0.423	0.675

Limit of Detection and Limit of Quantification:

Limits of detection and quantification The LOD and LOQ for the Known impurities and eszopiclone were estimated as the amounts for which the signal-to-noise ratios were 3:1 and 10:1, respectively, by injecting a series of dilute solutions of known concentration. The precision was also determined at the LOQ level by the analysis of six individual preparations of the four impurities and calculating the relative standard deviation (RSD; %) of the peak area for each impurity.

Linearity:

Linearity was established by analyzing six concentrations of eszopiclone and all impurities ranging between LOQ and 150%of the permitted maximum level of the impurity, by plotting the peak area ratio against the corresponding concentration. The correlation coefficients, slopes and y-intercepts of the calibration plots are reported. Calibration plots for the known impurities were linear over the tested ranges. The correlation coefficients were 0.99 for all the components (Table No.3). These results show an excellent correlation between peak area and concentration for their known impurities.

Table No.3: Linearity range was LOQ to 150% (with respect to 0.2% specification level)

Parameter	Eszopiclone	Impurity A	Impurity B	Impurity C	Impurity D
LOQ	0.65	0.58	0.32	0.31	0.67
LOD	0.16	0.14	0.08	0.10	0.15
Slope	153.162	168.132	205.282	281.891	125.132
Correlation	**0.998**	**0.999**	**0.99998**	**0.9996**	**0.9998**

Accuracy:

The studies were carried out at four different levels: LOQ, 50%, 100%, and 150% of limits. The percentage of recoveries of Imp-A Imp-B, Imp-C and Imp-D were calculated with respect to amount spiked and amount recovered. The percentage recovery at each level was calculated against the Eszopiclone standard. Mean recovery should be in the range of 90.0% to 110.0% for 50%, 100% and 150% levels and 85% to 115% for LOQ level. Mean recovery in percentage is reported in Table no. 4.

Table No. 4: Accuracy of Impurity of Eszopiclone Tablets

	Mean Recovery (%)			
Name of Impurity	**LOQ**	**Accuracy 50%**	**Accuracy**	**Accuracy**
Impurity A	89.6	92.5	95.2	96.7
Impurity B	96.5	101.4	101.9	101.8
Impurity C	99.8	97.2	98.6	99.8
Impurity D	96.7	98.2	99.6	105.2

Precision:

Precision is the closeness of agreement between a series of measurements obtained from multiple sampling of same sample under the prescribed conditions. Quantification of individual impurities and Eszopiclone Tablets was performed for each of the preparations and the percent relative standard deviation (RSD) was determined for the content of the impurities. To evaluate the intermediate precision, the same experiment was repeated with a different lot of column and a different instrument in the same laboratory. Precision data reported in table no.5.

Table No. 5: Over all %RSD Comparison for Impurities in Precision and Ruggedness study

Sr. No.	**% Impurity C**	**% Impurity D**	**% Impurity E**	**% Impurity E**	**% Unk Max**	**% Total Imp**
Precision	0.053	0.028	0.021	0.022	0.029	0.291
Precision	0.053	0.028	0.021	0.021	0.029	0.290
Precision	0.053	0.028	0.021	0.021	0.030	0.293
Precision	0.053	0.028	0.021	0.020	0.030	0.295
Precision	0.053	0.028	0.022	0.021	0.030	0.297
Precision	0.053	0.028	0.022	0.020	0.027	0.295
Rugge-1	0.052	0.027	0.022	0.022	0.029	0.294
Rugged-2	0.053	0.028	0.022	0.021	0.029	0.297
Rugged-3	0.053	0.028	0.022	0.021	0.029	0.296
Rugged-4	0.053	0.028	0.022	0.022	0.029	0.297
Rugged-5	0.053	0.027	0.022	0.021	0.029	0.295
Rugged-6	0.053	0.027	0.022	0.022	0.029	0.295
Mean	**0.053**	**0.028**	**0.214**	**0.211**	**0.029**	**0.295**
SD	**0.000**	**0.000**	**0.002**	**0.0054**	**0.001**	**0.002**
% RSD	**0.000**	**0.000**	**0.93**	**2.54**	**3.45**	**0.68**

Robustness:

To determine the robustness of the method, the experimental conditions were deliberately changed and the resolution of eszopiclone and their known impurities was evaluated. To study the effect of flow rate on resolution, it was changed to 1.4 and 1.6 mL/min. The effect of pH was studied at pH 3.3 and 3.7. The effect of column temperature was studied at 35 and 45°C. In all of these experiments, the mobile phase components were not changed. The effect of the percent organic strength on resolution was studied by varying acetonitrile by –10 to 10% while the other mobile phase components was held constant. In all deliberately varied chromatographic conditions, the selectivity and performance of the method were unchanged, which proves the robustness of the method.

Stability of Analytical solution:

The solution stability of sample and standard solution provide an indication of the method's reliability in normal usage during the storage of the solutions used in the method. No significant changes were experienced in the content of any of the impurities during solution stability. The results from solution stability and mobile phase stability experiments confirmed that the standard solutions and sample were stable for up to 60 hrs during the determination of related substances. The mobile phase was stable up to 60 hrs.

Summary of system suitability

System suitability was evaluated by injecting Standard solution during different days of validation and monitoring tailing factor and theoretical plates for different parameters .The % relative standard deviation for the peak area counts of Eszopiclone from five replicate injections of standard solution was verified at every stage. Results are tabulated in Table 6.

Table No.6: Table for System Suitability

Sr No	Name of Experiment	Theoretical Plate	Tailing Factor	%RSD
1	System precision, Method Precision, Solution Stability	11528	1.1	0.78
2	Linearity	12536	1.1	1.25
3	Accuracy (Robustness IFR,DFR,ICT,DCT,IPH,DPH)	12458	1.1	2.26
4	Ruggedness	10253	1.2	3.33
5	Specificity / Force Degradation	18252	1.0	0.89

Summary and Conclusion:

The Validated HPLC method for related substance of eszopiclone is linear, precise, accurate and specific. The results of the validation carried out for the method satisfied the ICH requirements. This method can be used for the detection and quantification of known, unknown and degradation impurities in the eszopiclone tablets during routine analysis and also for stability studies in view of its capability to separate degradation products.

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List of Abbreviations:

No. Number
LOQ Limit of Quantitation
LOD Limit of Detection
Imp Impurity
Unk Unknown
Max Maximum
Hrs Hours
HPLC High performance Liquid Chromatography
RSD Relative Standard Deviation
RRT Relative retention time

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