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Quantification and Validation of Eszopiclone by Liquid Chromatographic Method Through Isocratic Separation

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KEYWORDS

Eszopiclone, RP-HPLC, VWD detector, ICH guidelines.

ABSTRACT:

Introduction: A reverse phase high performance liquid chromatographic (RP-HPLC) method was developed and validated for the estimation of Eszopiclone in pharmaceutical dosage forms.

Objectives: The chromatographic separation of Eszopiclone was achieved on a Thermo Hypersil BDS C18 column (250mm×4.6 mm, 5µm particle size), Agilent LC1220 HPLC system with UV detection (VWD detector) at 315nm.

Methods: The optimized mobile phase was consisted of Methanol: Water (PH adjusted to 2.5 with orthophosphoric acid) (40:60 v/v). The flow rate was 1ml / min and effluents were monitored at 315nm. Chromatogram showed the main peak at a retention time of 2.057min. The method was validated for linearity, accuracy, precision, and limit of detection, limit of quantitation, robustness and ruggedness.

Results: The linearity was found in the concentration range of 25-150µg/ml. The Correlation coefficient was 0.999. The regression equation was found to be Y = 71703x+16358. The limit of detection and limit of quantitation for estimation of Eszopiclone was found to be 0.05 µg / ml and 0.16 µg / ml respectively.

Conclusions: Recovery of Eszopiclone was found to be in the range of 99.9-100.01%. Proposed method was successfully applied for the quantitative determination of Eszopiclone in pharmaceutical dosage forms as per ICH guidelines.

1. Introduction:

Eszopiclone is a nonbenzodiazepine hypnotic agent used in the treatment of patients with insomnia [1]. Eszopiclone is chemically known as [(7S)-6-(5-chloropyridin-2-yl)-5-oxo-7H-pyrrolo [3, 4-b] pyrazin-7-yl] 4-methylpiperazine-1-carboxylate was shown in (Figure 1).

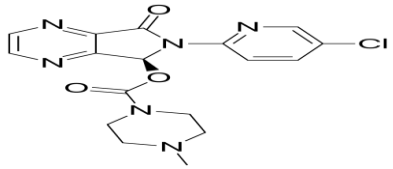

Fig. 1. Chemical structure of Eszopiclone

Literature review tells that very few analytical methods have been reported for the determination of Eszopiclone which includes UV-Spectrophotometry [2-6], High performance liquid chromatography [7-8] and Liquid chromatography-Mass

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spectroscopy method [9-11]. The present study was aimed to develop a novel, simple, economic and validated RP-HPLC method for the estimation of Eszopiclone according to ICH guidelines [12].

2. Materials And Methods:

Chemicals and Reagents:

Eszopiclone bulk drug were kindly provided as gift sample by Emcure Pharmaceuticals Limited, India. Orthophosphoric acid (Merck Chemical Company, GR-Grade), Water (Merck Chemical Company, HPLC-Grade) and Methanol (Merck Chemical Company, HPLC-Grade) were used in the study. Zolnite® tablet contain Eszopiclone 2mg is obtained from a local pharmacy manufactured by Sun Pharmaceuticals Industries Ltd, India.

Instrumentation:

The chromatography was performed on Agilent LC1220 HPLC system, equipped with VWD detector and EZ Chrome software, Thermo Hypersil BDS C_{18} column (250mm×4.6 mm, 5µm particle size) was used as stationary phase. All weights were taken on electronic balance (Model: CA123, Make: Contech), pH Meter (Model: 3 Star, Make: Global) and Sonicator (Model: UCB 70, Make: Life care) were used in the study.

Chromatographic conditions:

In this work a reverse phase Thermo Hypersil BDS C_{18} column (250mm×4.6 mm, 5µm particle size) was chosen as stationary phase and mobile phase consisting of mixture of Methanol: Water (P^H adjusted to 2.5 with orthophosphoric acid) (40:60 v/v) was delivered at a flow rate of 1.0ml/min and detector wavelength at 315 nm. Injection volume was 20µl. The run time was 5min and the retention time of Eszopiclone was found to be 2.057min.

Chromatographic Parameters:

Equipment : Agilent LC1220 HPLC system, equipped with VWD detector

Column : Thermo Hypersil BDS C_{18} column (250mm×4.6 mm, 5µm particle size)

Flow rate : 1ml/min

Wavelength : 315 nm

Injection volume : 20 µl

Column oven : Ambient

Run time : 5 Minutes

Preparation of mobile phase:

Solution A: 1000ml of HPLC grade water was degassed in ultrasonic water bath and filtered through 0.45µm filter using vacuum filtration and the pH was adjusted to 2.5 with orthophosphoric acid.

Solution B: Methanol HPLC-Grade

Mobile Phase: Volume of Solution (A) and solution (B) taken in ratio 60:40 (v/v) and mixed well and filtered through 0.45µm membrane filter and degas for 10 minutes.

Preparation of diluent:

Mobile phase was used as diluent.

Preparation of Standard Stock Solution:

An accurately weighed quantity of Eszopiclone 100mg was transferred to 100ml volumetric flask, dissolved in 100ml distilled water, the final volume was made with distilled water to obtain standard solution having concentration of 1000µg/ml. These stock solutions were used to prepare further dilutions.

Preparation of Sample Solution:

Eszopiclone is available as tablets containing 2mg of Eszopiclone. Eszopiclone is available in the local market with brand names Zolnite® (2mg, Sun Pharmaceuticals Industries Ltd, India). Twenty tablets of Eszopiclone were taken and made into a fine powder of the tablets and the powder equivalent to 10mg of Eszopiclone was weighed accurately and transferred into a 100ml standard volumetric flask. The contents were dissolved in mobile phase and sonicated for 30 Minutes. This entire solution was filtered through 0.45 micron Whatmann filter paper (No. 41) and the final solution was made with mobile phase to get the solution of 100µg/ml. 5ml of the solution were pipetted out separately into 10ml volumetric flask and make up to the mark to give 50µg/ml concentration. The sample solution 20µl was injected and chromatographed and the peak areas were measured for Eszopiclone was shown in (Figure 2 and 3) respectively.

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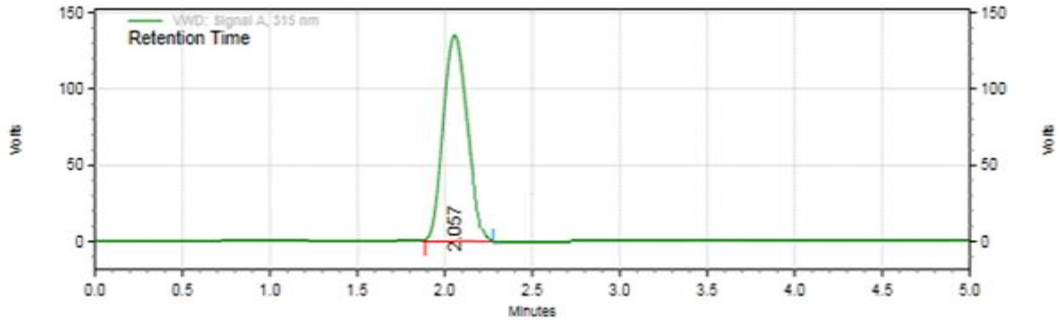

Fig. 2. Standard chromatogram of Eszopiclone

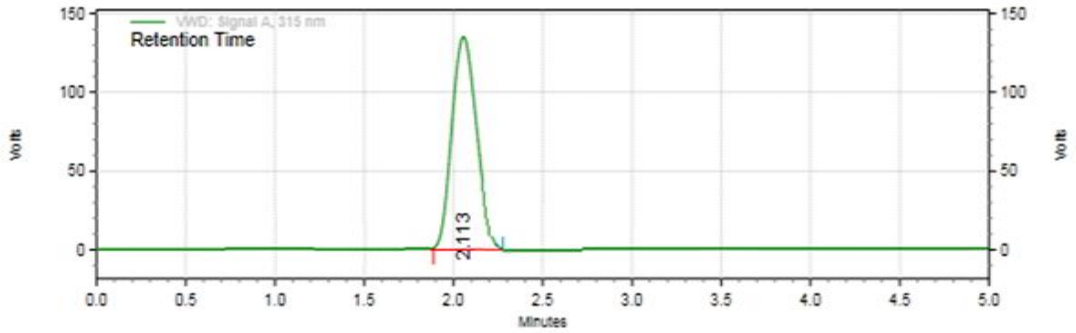

Fig. 3. Sample chromatogram of Eszopiclone

The % Assay was calculated by comparing the peak area of standard and sample chromatogram by using the formula given below and the assay result was shown in (Table 1).

$$\text{Assay \%} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{\text{Avg. Wt}}{\text{Label Claim}} \times 100$$

Where:
AT = Average peak area of sample preparation
AS= Average peak area of standard preparation
WS = Weight of standard taken in mg
WT=Weight of sample taken in mg
P = Percentage purity of working standard
DS= Dilution factor for standard preparation
DT=Dilution factor for sample preparation

Table 1. Assay of marketed formulation of Eszopiclone

Drug	Zolnite® Label Claim (mg)	Amount Found (mg)	% Label Claim ± % RSD (n=3)
Eszopiclone	2	1.99	99.5±0.3

3. Results:

Optimization of RP-HPLC method

For the method optimization, different mobile phases were tried, but acceptable retention times, theoretical plates and good resolution were observed with Methanol: Water (P^H adjusted to 2.5 with orthophosphoric acid) (40:60 v/v) using Thermo Hypersil BDS C_{18} column (250mm×4.6 mm, 5µm particle size).

System Suitability:

At first the HPLC system was optimized as per the chromatographic conditions. One blank followed by six replicates of a single calibration standard solution of 50µg/ml of Eszopiclone was injected to check the system suitability. To ascertain the system suitability

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for the proposed method, the parameters such as retention time, theoretical plates and peak asymmetry were taken and results were presented in (Table 2).

Table 2. System suitability test parameters for Eszopiclone

Parameter (n=6)	Eszopiclone
Retention Time (Mins)	2.057
Theoretical plates	3645
Tailing factor	1.1

Specificity:

The effect of excipients and other additives usually present in the dosage form of Eszopiclone in the determination under optimum conditions was investigated. The specificity of the RP-HPLC method was established by injecting the blank and placebo solution into the HPLC system. The representative chromatogram of blank and placebo was shown in (Figure 4 and 5) respectively.

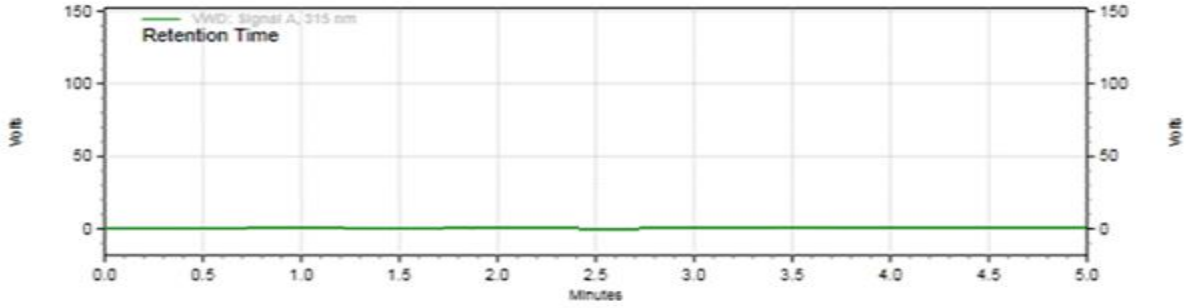

Fig. 4. Chromatogram of Blank

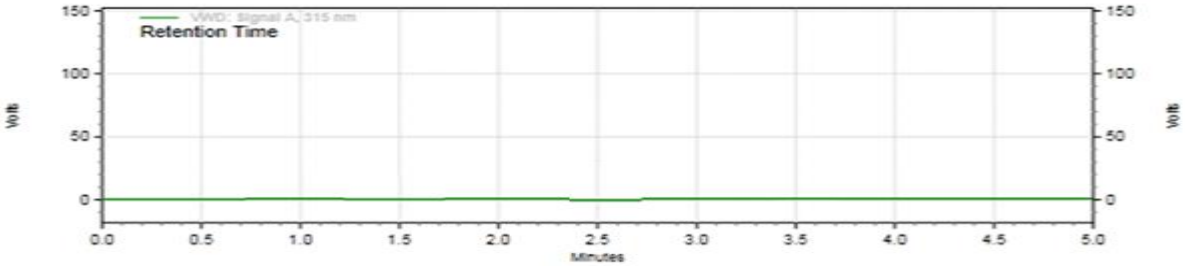

Fig. 5. Chromatogram of Placebo

Linearity:

Linearity was performed by taking from stock solution (1000µg/ml) aliquots of 0.25, 0.5, 0.75, 1, 1.25, 1.5 ml were taken in 10ml volumetric flasks and diluted up to the mark with diluent such that the final concentrations are in the range of 25-150 µg/ml. Each of these drug solutions (20µl) was injected into the chromatographic system for three times. The peak area and retention time were recorded and the mean values of peak areas were plotted against concentrations. The linearity data is presented in (Figure 6) and (Table 3). Acceptance Criteria: Correlation coefficient should be not less than 0.999.

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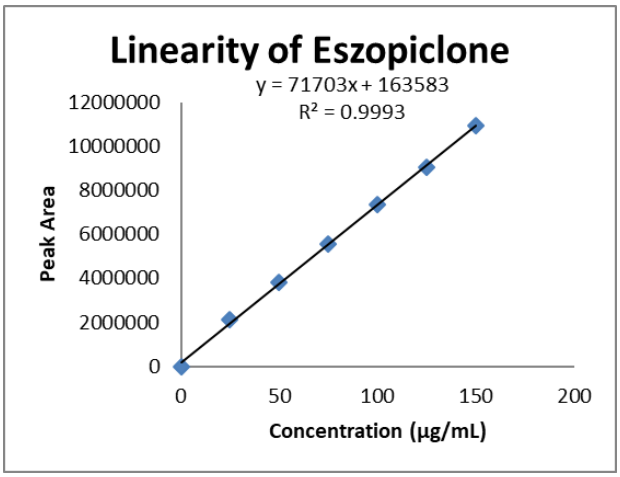

Fig. 6. Linearity graph of Eszopiclone

Table 3. Linearity data for Eszopiclone

Linearity of Eszopiclone	
Concentration (µg/ml)	**Peak Area**
25	2116519
50	3808144
75	5536950
100	7344093
125	9060775
150	10922444

Accuracy studies:

The accuracy of the method was determined by calculating recovery of Eszopiclone by the method of standard addition. Known amount of standard solution of Eszopiclone at 50%, 100% and 150% was added to a pre quantified sample solution and injected into the HPLC system. The mean percentage recovery of Eszopiclone at each level was calculated and the results were presented in (Table 4). Acceptance Criteria**:** The % Recovery for each level should be between 98.0 to 102.0%.

Table 4. Recovery study data of Eszopiclone

Sample name	Amount added (µg/ml)	Amount found (µg/ml)	% Recovery	Statistical Analysis
S_1:50%	25	24.87	99.48	Mean-99.96
S_2:50%	25	25.03	100.12	S.D-0.42
S_3:50%	25	25.07	100.28	%RSD-0.42
S_4:100%	50	49.78	99.56	Mean-99.9
S_5:100%	50	50.02	100.04	S.D-0.3
S_6:100%	50	50.05	100.10	%RSD=0.3
S_7:150%	75	74.98	99.97	Mean-100.01
S_8:150%	75	75.01	100.01	S.D-0.03
S_9 :150%	75	75.03	100.04	%RSD-0.03

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Precision studies for Eszopiclone:

Method precision (Repeatability):

Twenty tablets were accurately weighed and tablet powder equivalent to 10mg of Eszopiclone were taken into 100ml clean dry volumetric flask, diluent was added and sonicated to dissolve it completely and volume was made up to the mark with the same diluent and filtered through 0.45 µm nylon membrane filter. Further pipette out 5ml from the above Eszopiclone sample stock solution into a 10ml volumetric flask and diluted up to the mark with diluent to get the concentration of 50µg/ml of Eszopiclone. A homogenous sample of a single batch analysed six times and was checked whether the method is giving consistent results. The %RSD for the area of six replicate injections was calculated as mentioned in (Table 5). Acceptance Criteria: The % RSD for the peak area of six sample injections should not be more than 2%.

Table 5. Method precision data for Eszopiclone

Eszopiclone		
S.No.	Concentration (µg/ml)	% Assay
1	50	101.5
2	50	99.65
3	50	100.04
4	50	100.32
5	50	101.71
6	50	100.01
Average		100.54
SD		0.86
%RSD		0.85

System precision:

The system precision was carried out to ensure that the analytical system is working properly. The standard preparation concentration of 50µg/ml of Eszopiclone was injected six times into the HPLC and the %RSD for the area of six replicate injections was calculated as mentioned in (Table 6). Acceptance Criteria: The % RSD for the peak area of six standard injections should not be more than 2%.

TABLE 6. System precision data for Eszopiclone

Eszopiclone		
S.No.	Concentration (µg/ml)	Peak Area
1	50	3892645
2	50	3914582
3	50	3906594
4	50	3848246
5	50	3812278
6	50	3850362
Average		3870785
SD		36575.26
%RSD		0.94

Intermediate precision/ruggedness:

The intermediate precision (also known as Ruggedness) of the method was evaluated by performing precision on different laboratory by different analyst and different days. The sample preparation concentration of 50µg/ml of Eszopiclone was injected six times into the HPLC and the %RSD for the area of six replicate injections was calculated as mentioned in (Table 7). Acceptance Criteria: The % RSD for the peak area of six standard injections should not be more than 2%.

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TABLE 7. Ruggedness data for Eszopiclone

Ruggedness Data for Eszopiclone								
Laboratory-1 (% Assay)-HPLC-1					Laboratory-2 (% Assay)-HPLC-2			
	Analyst-1		Analyst-2		Analyst-1		Analyst-2	
Conc. (µg/ml)	Day-1	Day-2	Day-1	Day-2	Day-1	Day-2	Day-1	Day-2
50	101.9	100.00	99.75	100.53	99.66	100.48	100.13	100.98
50	100.73	99.82	100.36	99.97	99.97	99.97	99.49	99.68
50	100.04	100.72	101.08	100.39	100.29	99.38	100.54	100.09
50	100.00	99.99	99.98	99.74	100.85	100.86	100.84	100.60
50	101.33	100.52	100.53	100.77	100.04	99.59	99.78	100.20
50	101.05	99.85	100.06	100.09	100.47	101.01	100.35	99.75
Average	100.84	100.15	100.29	100.25	100.21	100.22	100.19	100.22
SD	0.74	0.38	0.47	0.38	0.42	0.67	0.5	0.5
%RSD	0.74	0.38	0.47	0.38	0.42	0.67	0.5	0.5
Intermediate precision within-laboratories variations (n=24)								
Laboratory-1 (% Assay)-HPLC-1					Laboratory-2 (% Assay)-HPLC-2			
Average	100.38				**Average**	100.21		
SD	0.17				**SD**	0.01		
%RSD	0.17				**%RSD**	0.01		
Reproducibility between laboratories (n=48) (% Assay)								
Average	100.3							
SD	0.12							
%RSD	0.12							

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

Limit of Detection (LOD) and Limit of Quantification (LOQ) were calculated as 3.3×SD/S and 10×SD/S respectively as per ICH guidelines, Where SD is the standard deviation of the response (Y-intercept) and S is the slope of the calibration curve. The LOD is the smallest concentration of the analyte that gives a measurable response (signal to noise ratio of 3). The LOD of Eszopiclone was calculated and shown in (Table 8). The LOQ is the smallest concentration of the analyte which gives response that can be accurately quantified (signal to noise ratio of 10). The LOQ of Eszopiclone was calculated and shown in (Table 8).

Table 8. Summary of validation parameter for Eszopiclone

Parameters	**RP-HPLC method**	
	Eszopiclone	
Linearity range (µg/ml)	25-150	
Slope	71703	
Intercept	16358	
Correlation coefficient	0.999	
LOD (µg/ml)	0.05	
LOQ (µg/ml)	0.16	
Method Precision (% RSD, n=6)	0.85	
System precision (% RSD, n=6)	0.94	
Ruggedness (% RSD, n=24)	Lab-1	Lab-2

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	0.17	0.01
Reproducibility (% RSD, n=48)	0.12	
% Accuracy	99.9-100.01	
Robustness (% RSD, n=6)	Less Flow rate	More Flow rate
	0.05	0.16
	Less Organic phase	More Organic phase
	0.16	0.87

Robustness:

As part of the Robustness, deliberate change in the flow rate and mobile phase proportion of ±10% was made to evaluate the impact on the method. The results reveal that the method is robust. The results are summarized in (Table 9).

Table 9. Summary of Robustness (Change in Flow Rate and mobile phase) for Eszopiclone

Parameters	Mean peak Area(n=3)	S.D	%R.S.D	R_T	Theoretical plates
Flow rate 0.9ml/min	4194199	19354.90	0.05	2.720	3567
Actual flow rate 1ml/min	**3901171**	**133695.6**	**0.34**	**2.057**	**3645**
Flow rate 1.1ml/min	3697370	58467.46	0.16	1.973	3734
10% less organic (64:36)	4200517	67750.37	0.16	2.70	4536
Actual mobile phase (60:40)	**3901171**	**133695.6**	**0.34**	**2.057**	**3645**
10% more organic (56:44)	3273557	286466.6	0.87	1.99	3927

4. Discussions:

To optimize the RP-HPLC parameters, several mobile phase compositions were tried. A satisfactory separation and good peak symmetry for Eszopiclone were obtained with a mobile phase containing a mixture of Methanol: Water (P^H adjusted to 2.5 with orthophosphoric acid) (40:60 v/v) was delivered at a flow rate of 1ml/min to get better reproducibility and repeatability. Quantification was achieved with VWD detection at 315nm based on peak area. The retention time of Eszopiclone was found to be 2.057min. Linearity was established for Eszopiclone in the range of 25-150µg/ml with correlation coefficient 0.999 and mean accuracies were found to be is 99.9% to 100.01% for Eszopiclone, which indicates accuracy of the proposed method. The % RSD values of accuracy for Eszopiclone were found to be < 2 %. The % RSD value of method precision was 0.85% for Eszopiclone and % RSD value of system precision was 0.94% for Eszopiclone. The % RSD value of reproducibility is 0.12% for Eszopiclone reveal that the proposed method is precise. LOD value for Eszopiclone was found to be 0.05µg/ml and LOQ value for Eszopiclone were found to be 0.16µg/ml. The % RSD values of robustness studies were found to be < 2% reveal that the method is robust enough. These data show that the proposed method is specific and sensitive for the determination of Eszopiclone.

5. Conclusion:

RP-HPLC method for the estimation of Eszopiclone in their bulk and pharmaceutical dosage form was established and validated as per the ICH guidelines. Linearity was achieved for Eszopiclone in the range of 25-150µg/ml with correlation coefficient 0.999. The percentage recovery of drug was achieved in the range of 98-102% which was within the acceptance criteria. The percentage RSD was NMT 2 % which proved the

precision of the developed method. The developed method is simple, sensitive, rapid, linear, precise, rugged, accurate, specific, and robust. Hence it can be used for the routine analysis of Eszopiclone in their bulk and pharmaceutical dosage form.

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