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Research Article

DEVELOPMENT AND VALIDATION OF AN RP-HPLC METHOD FOR THE DETERMINATION OF THE ESZOPICLONE IN TABLET DOSAGE FORMS

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ABSTRACT

A new simple, reliable, inexpensive, and accurate method was developed for the quantification of Eszopiclone in pharmaceutical dosage form. The developed method is an attempt to surpass the disadvantages associated with the reported methods, namely, less sensitive and tedious in usage for routine purposes. The separation was achieved on Purospher® Star RP18e,(150 x 4.6 mm; 5µ)in isocratic elution mode with the mobile phase consisting of 8.1 g of Sodium lauryl sulphate and 1.6 g Sodium dihydrogen phosphate monohydrate was dissolved in 1000 mL of Milli-Q water and pH was adjusted to 3.5 and acetonitrile in the ratio of 50: 50 (v/v) and the column was maintained at 30°C. The detection of eluent from the column was detected using photo diode array detector (PDA) at 303nm and the flow rate was maintained at 1.5 ml/min. Stability indicating assay method was developed and validated as per the ICH guidelines using various parameters, for example, accuracy, precision, limit of quantification, limit of detection, robustness, ruggedness, solution stability, recovery, forced degradation (hydrolysis, photo degradation, thermal degradation, and oxidation), and so forth. Percent relative standard deviation associated with all the parameters was less than 2, showing compliance with the acceptance criteria of ICH guidelines. The developed method was very sensitive as limit of quantification and limit of detection were found to be 0.132 µg/mL and 0.054 µg/mL, respectively. Forced degradation studies of drug reveal good stability under the chosen experimental conditions. As the proposed method could effectively separate the drug from its degradation products, it can be employed as stability-indicating method for the determination of the drugs in bulk and commercial products.

Keywords: Eszopiclone, Stability-indicating assay, Isocratic elution, RP-HPLC method.

INTRODUCTION

Eszopiclone is a nonbenzodiazepine hypnotic which is slightly effective for insomnia[1]. (Difficulty falling asleep or staying asleep). Eszopiclone is the active dextrorotatory stereoisomer of zopiclone, and belongs to the class of drugs known as cyclopyrrolones. Eszopiclone is in a class of medications called hypnotics. It works by slowing activity in the brain to allow sleep. Eszopiclone acts on benzodiazepine binding site situated on $GABA_A$ neurons as an agonist. Eszopiclone is rapidly absorbed after oral administration, with serum levels peaking between 1 and 1.3 hours. The elimination half-life of eszopiclone is approximately 6 hours and it is extensively metabolized by oxidation and demethylation. Approximately 52 to 59% of a dose is weakly

bound to plasma protein. Cytochrome P_{450} (CYP) isozymes $CYP3A_4$ and $CYP2E_1$ are involved in the biotransformation of eszopiclone; thus, drugs that induce or inhibit these CYP isozymes may affect the metabolism of eszopiclone. Less than 10% of the orally administered dose is excreted in the urine as racemic zopiclone. Eszopiclone,(S)-6-(5-Chloro-2-pyridinyl)-7-oxo-6,7-dihydro-5H-yrrolo[3,4b]pyrazin5-yl-4-methyl-1-piperazinecarboxylate,is a white to light-yellow crystalline solid, very slightly soluble in water, slightly soluble in ethanol, soluble in phosphate buffer.

Literature review reveals that some analytical methods have been reported for Eszopiclone by UV[2] and difference spectroscopic methods, in biological fluids using LCMSMS[3-5],HPLC[6-15] validated LC method for the estimation of Eszopiclone in bulk and tablet dosage form[16-18], stability indicating RP-LC method[19].Proposed method was validated as per ICH guidelines[20-22] and its updated international convention .As in pharmaceutical industry, time and expense play a very crucial role. Pharmaceutical industries always admire simple and more sensitive methods for routine usage. Therefore, a need of simple, reliable, inexpensive, and accurate stability indicating method for analysis of Eszopiclone as bulk or as pharmaceutical dosage forms has always been felt. The present study was aimed to develop and validate stability indicating method for the quantification of eszopiclone in pharmaceutical dosage forms.

EXPERIMENTAL

Chemicals, Reagents and Solutions

The author had attempted to develop a liquid chromatographic method for estimation of Eszopiclone. The separation of the analyte was done by using an isocratic Waters Allaince HPLC instrument; on a Purospher® Star RP18e column (150 x 4.6 mm; 5µ).The instrument was equipped with a pump (2695), injector, PDA Detector (2996) and column oven. Data acquisition was done by using Empower software.

Degassing of the mobile phase was done by using a Spectra lab model DGA 20A3 ultrasonic bath sonicator. A Sartorious electronic balance was used for weighing the materials. Class 'A' Borosil glassware was employed for volumetric and general purpose in the study. The reference sample of Eszopiclone was gifted by M/s Lupin Pharmaceutical Limited, Pune. The tablets of Lunesta (The branded formulation of Sepracor) were procured from the local market. Sodium lauryl sulphate (Merck), Sodium dihydrogen phosphate monohydrate(GR grade), *Ortho* Phosphoric Acid (min. 88%), Acetonitrile(HPLC grade),water(Milli-Q / HPLC grade).

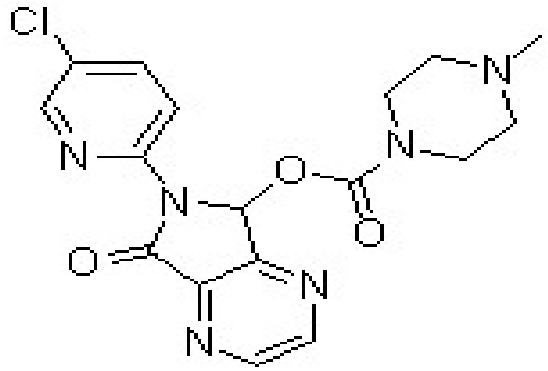


Fig. 1:Chemical structure of Eszopiclone

Chromatographic Conditions

Preparation of 10% Ortho Phosphoric Acid solution

Diluted 5mL Ortho Phosphoric Acid (min. 88%) to 50mL with Milli-Q water.

Buffer solution

Sodium lauryl sulphate of 8.1g and 1.6 g Sodium dihydrogen phosphate monohydrate was dissolved in 1000 mL of Milli-Q water. The pH was adjusted to 3.5 ± 0.05 with 10% Ortho phosphoric acid solution. This solution was filtered through a 0.45µm membrane filter.

Mobile Phase

The buffer solution and acetonitrile were mixed in the ratio of 50: 50 (v/v), filtered and degassed.

Diluent

The above mobile phase was used as a diluent also in preparing drug solutions.

Optimized Chromatographic Conditions

Parameter	Value
Column	Purospher® Star RP18e,(150 x 4.6 mm; 5µ)
Mobile Phase	Phosphatebuffer:acetonitrile (50:50)
Flow Rate	1.5 mL/min
Run Time	10 min
Column Temperature	30±1 ˚C
Volume of Injection	20 µL
Detection Wave Length	303 nm
Retention Time	4.80 min

Preparation of standard Stock solution

About 50 mg standard was accurately weighed and transferred into 50 mL volumetric flask added about 30 mL of the diluent and

sonicated to dissolve. Diluted to volume with the diluent and mixed well.

Preparation of working standard solution

From the standard stock solution 5.0 mL was diluted to 50 mL with the diluent to give a working standard solution containing 100 µg/mL. The solution was filtered through a 0.45µm Nylon membrane filter.

Formulation sample solution preparation

Ten tablets of Eszopiclone (Lunesta Tablets of Sepracor Inc) were taken into a 100 mL volumetric flask, about 60 mL of the diluent was added and the contents were sonicated for 10 min with intermittent shaking. The flask was cooled to room temperature and the solution was made up with the diluents and mixed well. The solution was filtered through a 0.45µ Millipore nylon membrane filter.

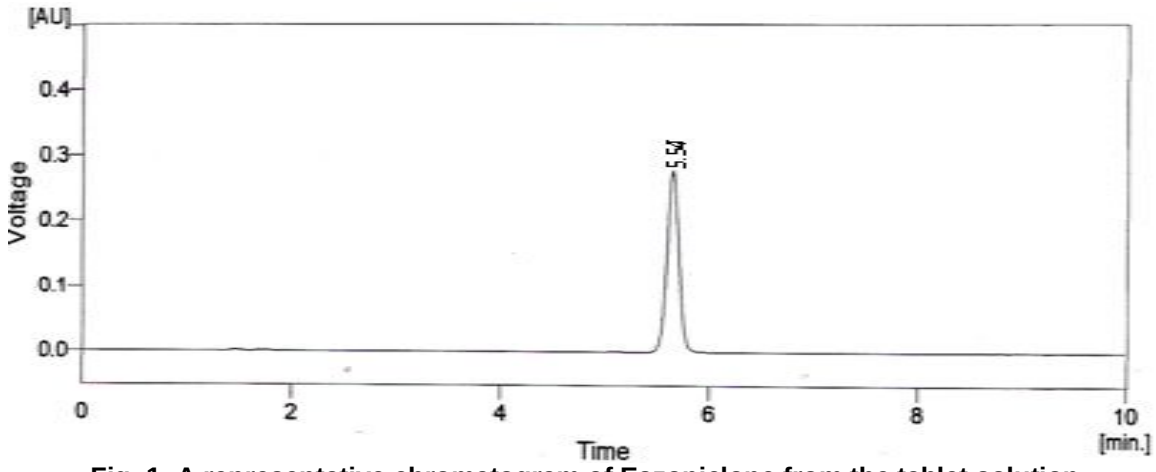


Fig. 1: A representative chromatogram of Eszopiclone from the tablet solution

Method Validation

The method was validated in compliance with ICH guidelines[11-15]. The parameters determined for validation were specificity, precision, accuracy, robustness, linearity, forced degradation, Limit of Quantification and Limit of Detection, system suitability and stability of analytical solution.

1. Specificity

The method specificity was assessed by comparing the chromatograms obtained from a placebo solution containing a mixture of most commonly used excipients without the drug and another solution containing the excpeints with the drug. These solutions were prepared in the diluent. The drug to excipient ratio used was similar to that in the commercial formulation. The mixtures were filtered through 0.45µ membrane filter before injection. The placebo solution and the sample solution (placebo and the drug) were injected into HPLC system separately in triplicate and the relevant chromatograms observed. There was no interference from blank and placebo at the retention time of analyte peak. The absence of additional peaks in the chromatogram indicates non interference of the commonly used excipients in the tablets and hence the method is specific.

2. Forced degradation studies

Forced degradation study was carried out by treating placebo and the formulation sample to the following conditions.

1. Treatment with hydrochloric acid.
2. Treatment with sodium hydroxide.
3. Treatment with hydrogen peroxide.
4. Thermal exposure.
5. Photolytic exposure.
6. Exposure to humidity.

Acid degradation

The Placebo and the sample solution of Eszopiclone tablets were transferred separately into 100 mL volumetric flasks. 60 mL of diluent was added to each flask and sonicated for 10 min with intermittent shaking. To each flask 5mL of 5N hydrochloric acid was added and the solutions were kept in water bath at 80°C.After 5 min flasks were removed from water bath and cooled to room temperature. The resulting solutions were neutralized using 5.0 mL of 5N sodium hydroxide. Treated placebo and sample solutions were analyzed as per the test method.

Alkali degradation

The Placebo and the sample solution of Eszopiclone tablets were transferred into 100 mL volumetric flasks. 60 mL of the diluent was added to each flask and sonicated for 10 min

with intermittent shaking. To each flask, 1mL of 0.1N sodium hydroxide was added. Then the resulting solutions were neutralized by using 1.0 mL of 0.1N hydrochloric acid. The Treated placebo and sample solutions were analyzed as per the test method.

Peroxide degradation

The Placebo and the sample solution of Eszopiclone tablets were transferred into 100 mL volumetric flasks. 60 mL of diluent was added to each flask and sonicated for 10 min with intermittent shaking. To each flask 5mL of 30% v/v solution of hydrogen peroxide was added and the solutions were kept on bench top for 1 hr at room temperature. The treated placebo and sample solutions were analyzed as per the test method.

Thermal degradation

The Placebo and the sample solution of Eszopiclone tablets were transferred into 100 mL volumetric flasks were exposed to heat in oven at 80°C for about 24 hours. After 24 hr cooled to room temperature and 60 mL of diluent was added to each flask and sonicated for 10min with intermittent shaking to each flask .The treated placebo and sample solutions were analyzed as per the test method.

Photolytic degradation

The Placebo and the sample solution of Eszopiclone tablets were exposed to UV and fluorescent light in Suntest apparatus for about 22 hr (1.2million lux hr). Treated placebo and sample solutions were analyzed as per the test method.

Humidity degradation

The Placebo and sample solution of Eszopiclone tablets were exposed to humidity at 40°C/75%RH about 115 hr. The treated sample and placebo solutions were analyzed as per the test method.

The % Assay values with respect to untreated sample and Peak Purity data of Eszopiclone at each condition are tabulated in Table 1. Fig 2-10 for chromatograms and purity plots of untreated and treated sample solutions respectively.

Table 1: Forced degradation data for Eszopiclone

S.No.	Condition	% Assay	% Degradation w.r.t. Untreated sample	Peak Purity		
				Purity Angle	Purity Threshold	Purity Flag
1	Untreated Sample	99.80	--	0.054	1.065	No
2	Acid Degradation	86.30	13.53	0.518	2.239	No
3	Alkali Degradation	94.28	5.53	0.055	1.066	No
4	Peroxide Degradation	81.06	18.78	0.228	1.377	No
5	Thermal Degradation	94.34	5.47	0.056	1.079	No
6	Photolytic Degradation	93.16	6.65	0.191	1.385	No
7	Humidity Degradation	94.56	5.25	0.068	1.113	No

3. Precision

3.1 System precision

Six replicates of standard solution of Eszopiclone were injected into HPLC system. % RSD of Eszopiclone peak area counts from six replicate injections of standard solution was less than 2.0 and it meets the acceptance criterion.

Table 3: System precision data

Standard solution	
S. No.	Eszopiclone peak area counts
1.	3128395
2.	3129545
3.	3128317
4.	3127253
5.	3124127
6.	3128613
Mean	3127708
SD	1900.9
% RSD	0.06

3.2 Method precision

Six sample preparations were made from a single batch of Eszopiclone tablets and analyzed as per the proposed method. The % Assay of Eszopiclone for six sample preparations was less than 2.0 and meets the acceptance criterion.

3.3 Intermediate precision (Ruggedness)

Ruggedness of method was verified by analyzing six sample preparations of same batch used under method precision as per proposed method by different analysts using different instrument and different column on different day. The amount of Eszopiclone in Eszopiclone tablets was determined. % RSD for % assay of Eszopiclone was calculated, for six preparations. % RSD for % assay of Eszopiclone was less than 2.0 and meets the acceptance criteria.Overall % RSD for % assay of Eszopiclone obtained from ruggedness and method precision was less than 2.0 and meets the acceptance criteria.

.**Table 4: precision data**

S. No.	Assay of Eszopiclone	
	Method precision	Ruggedness
1.	98.40	100.35
2.	99.14	99.96
3.	98.82	99.92
4.	99.27	100.38
5.	101.43	100.09
6.	101.74	99.75
Mean	99.80	100.08
SD	1.418	0.250
% RSD	1.42	0.25
Overall Mean	99.94	
Overall SD	0.981	
Overall %RSD	0.98	

4. Accuracy

The placebo was spiked with known amounts of Eszopiclone (API) at about 50%, 100% and 150% of test concentration of 1mg strength and 150% of test concentration of 3mg strength prepared in triplicate at each level. Amount of Eszopiclone was quantified and % recovery was calculated from amount found and actual amount added. % Recovery at each level was calculated. Analytical method meets acceptance criteria for recovery study. Hence the method is accurate and precise.

Table 5: Accuracy data

Spike level (%)	Actual Amount of Eszopiclone added in mg	Amount of Eszopiclone found in mg	%Recovery	Mean	SD	% RSD
50(strength 1mg)	49.84	50.21	100.74	100.74	0.020	0.02
	49.84	50.20	100.72			
	49.84	50.22	100.76			
100(strength 1mg)	99.68	100.78	101.10	100.37	0.633	0.63
	99.68	99.72	100.04			
	99.68	99.65	99.97			
150(strength 1mg)	149.52	149.11	99.73	99.45	0.361	0.36
	149.52	148.88	99.57			
	149.52	148.08	99.04			
150(strength 3mg)	179.42	181.97	101.42	101.14	0.250	0.25
	179.42	181.10	100.94			
	179.42	181.32	101.06			
Overall mean			**100.42**			
Overall SD			**0.732**			
Overall % RSD			**0.73**			

5. Linearity

Linearity of response was performed using the standard solution in a range of 49.85mcg/mL to 179.46mcg/mL [about 50% - 150% of the test concentration. The results are tabulated in Table 6 and represented graphically in refer Fig 11.

The relevant correlation coefficient value is more than 0.99.

Table 6: Linearity data

Spike level (%)	Concentration in mcg/mL	Eszopiclone peak area counts
50%	49.85	1514336
60%	71.78	2176569
80%	95.71	2904076
90%	107.68	3262037
100%	119.64	3597039
110%	131.60	3929293
120%	143.57	4326918
140%	167.50	5072146
150%	179.46	5416020
Slope		**30074**
Intercept		**12910**
Correlation coefficient (r)		**0.99990**

6. Stability in analytical solution

Stability of Eszopiclone in analytical solution was verified by analyzing sample solution initially and also at different time intervals up to 29 hrs and 10 min by storing sample solution at room temperature. Cumulative %RSD is less than 2.0 at each time interval.

Table 7: Solution stability data

Time	Eszopiclone peak area counts	Cumulative % RSD
Initial	3012118	-
00hr 10min	3014361	0.05
00hr 21min	3018255	0.10
00hr 32min	3023660	0.17
00hr 43min	3022004	0.16
00hr 54min	3028927	0.21
05hrs 16min	3058389	0.52
05hrs 27min	3054838	0.59
10hrs 01min	3067099	0.69
10hrs 11min	3075459	0.78
14hrs 46min	3110092	1.03
14hrs 56min	3098506	1.11
19hrs 30min	3113400	1.21
19hrs 41min	3105931	1.25
24hrs 15min	3136267	1.38
24hrs 26min	3143557	1.48
28hrs 59min	3156966	1.60
29hrs 10min	3174449	1.73

CONCLUSION

The solution was found to be stable up to 29 hrs at room temperature hence it is concluded that the proposed analytical method meets the pre-established acceptance criteria.

7. Robustness

To evaluate its robustness, following small deliberate variations were made in the method and the samples were analyzed in triplicate. Change in Flow rate by (±10%), Change in Organic content variation in mobile phase by (± 2% absolute), Change in pH of buffer for mobile phase by (± 0.1 unit), Change in Wavelength by (± 5 nm), Change in Column oven temperature by (± 5°C),System suitability was evaluated in each condition and results were compared with method precision results. The results obtained are tabulated in table 8.Overall %RSD is than 2.0 for individual experiment.

As method meets pre-established acceptance criterion, the method is considered to be robust for small changes in flow rate, pH of buffer for mobile phase, wavelength, organic content in mobile phase and column oven temperature.

Table 8: Robustness data

S.NO	M.P.	-Flow (1.35 mL/min)	+Flow (1.65 mL/min)	-pH (3.40 unit)	+pH (3.60 unit)	- nm (298 nm)	+nm (308 nm)	-Org -2 % absolute	+Org +2 % absolute	-Temp (25°C)	+Temp (35°C)
1	98.40	101.25	100.33	101.04	98.68	98.48	98.43	98.43	98.46	99.74	98.13
2	99.14	101.33	99.83	100.26	97.56	99.11	99.05	98.40	97.78	99.67	97.90
3	98.82	101.09	100.06	100.27	97.83	98.70	98.70	97.58	97.35	99.51	97.79
4	99.27	-	-	-	-	-	-	-	-	-	-
5	101.43	-	-	-	-	-	-	-	-	-	-
6	101.74	-	-	-	-	-	-	-	-	-	-
Overall mean		100.27	99.89	100.04	99.21	99.45	99.44	99.25	99.15	99.75	99.18
Overall SD		1.329	1.136	1.199	1.460	1.246	1.253	1.417	1.508	1.126	1.459
Overall % RSD		1.33	1.14	1.20	1.47	1.25	1.26	1.43	1.52	1.13	1.47

8. Limit of Detection and Limit of Quantification

Limit of detection (LOD) is defined as the lowest concentration of analyte that gives a measurable response. LOD is determined based on signal to noise ratio (S/N) of three times typically for HPLC methods. The limit of quantification (LOQ) is defined as the lowest concentration that can be quantified reliably with a specified level of accuracy and precision. It is the lowest concentration at which the precision expressed by an RSD of less than 2%. In this study the analyte response is 10 times greater than the noise response. For this study six replicates of the analyte at lowest concentration in the calibration range were measured and quantified. The LOD and LOQ of Eszopiclone obtained by the proposed method were 0.054 and 0.132 µg/mL respectively.

9. 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 9.

Table 9: Summary of system suitability data

S. No.	Name of Experiment	Theoretical plates	Tailing factor	%RSD
1	System precision, Method precision & Solution Stability	8426	1.1	0.07
2	Robustness (- Wavelength)	8422	1.1	0.11
3	Robustness (+ Wavelength)	8429	1.1	0.11
4	Linearity	7291	1.0	0.47
5	Recovery & Specificity	7242	1.0	0.27
6	Ruggedness	7402	1.1	0.22
7	Robustness (- Temperature)	7828	1.1	0.40
8	Robustness (+ Temperature)	8584	1.0	0.42
9	Robustness (- Flow)	8749	1.0	0.22
10	Robustness (+ Flow)	7794	1.0	0.16
11	Robustness (- pH)	7222	1.1	0.49
12	Robustness (+ pH)	7100	1.1	0.63
13	Robustness (- Organic)	6501	1.1	0.27
14	Robustness (+ Organic)	7164	1.0	0.25
15	Forced Degradation	8876	1.0	0.38

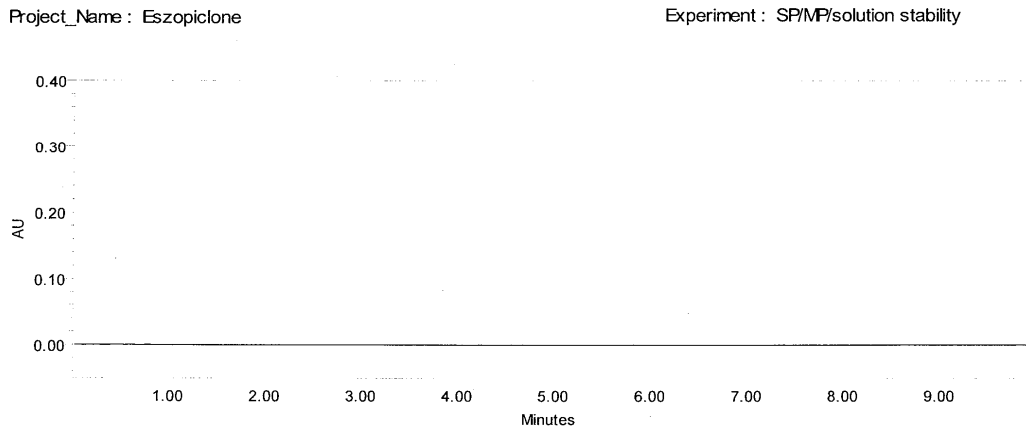


Fig. 2: HPLC Chromatogram of Blank

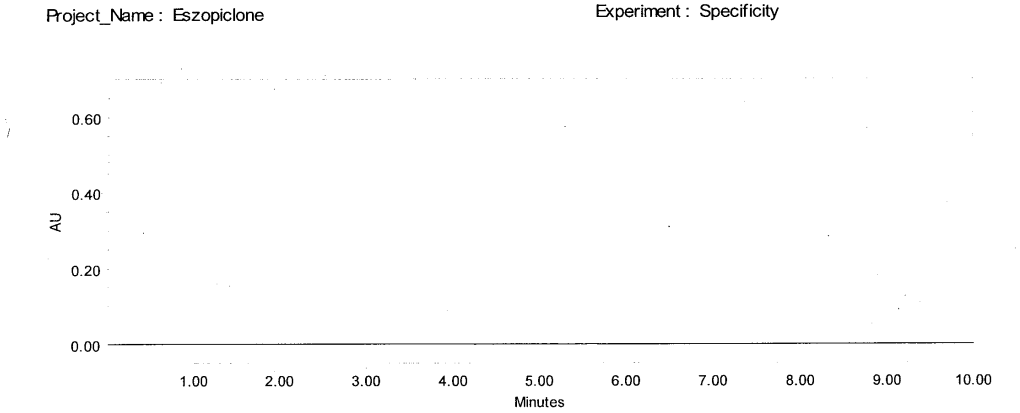


Fig. 3: HPLC Chromatogram of Placebo

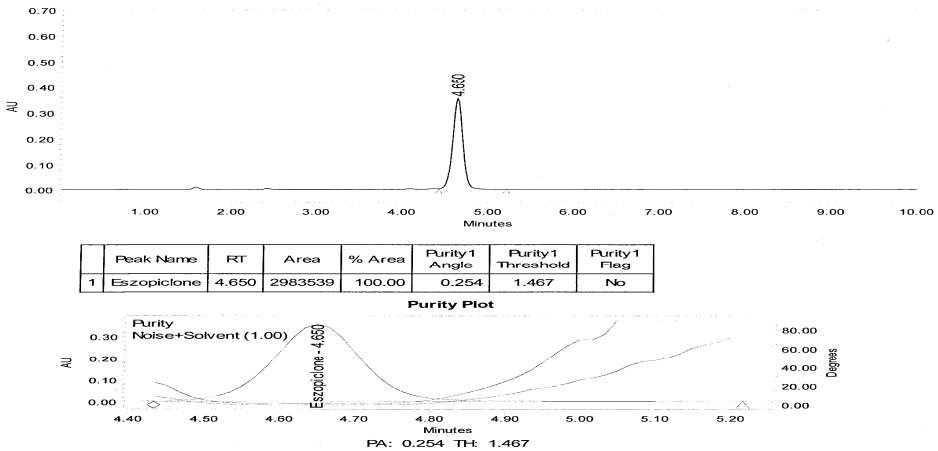
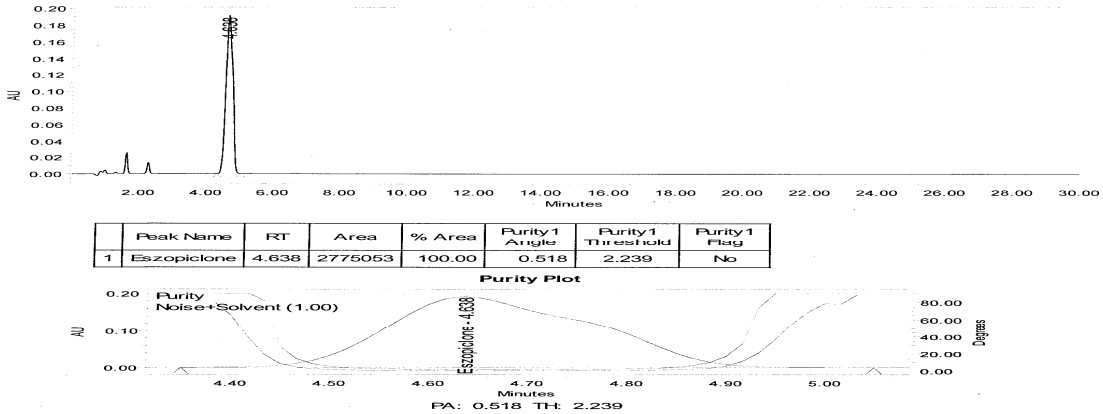


Fig. 4: HPLC Chromatogram of placebo with drug sample solution



	Peak Name	RT	Area	% Area	Purity1 Angle	Purity1 Threshold	Purity1 Flag
1	Eszopiclone	4.638	2775053	100.00	0.518	2.239	No

Fig. 5: HPLC Chromatogram and purity plot of Acid treated sample

	Peak Name	RT	Area	% Area	Purity1 Angle	Purity1 Threshold	Purity1 Flag
1	Eszopiclone	4.790	3029274	100.00	0.055	1.066	No

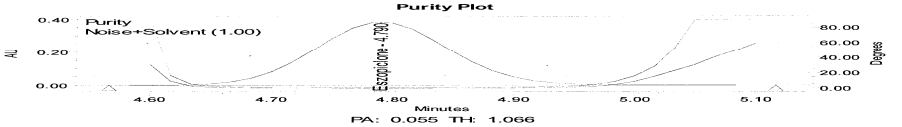
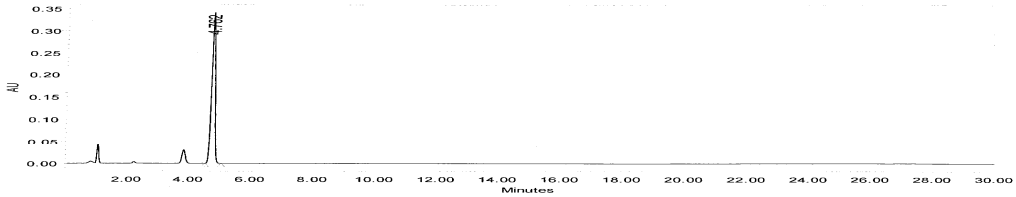


Fig. 6: HPLC Chromatogram and purity plot of Alkali treated sample



	Peak Name	RT	Area	% Area	Purity1 Angle	Purity1 Threshold	Purity1 Flag
1	Eszopiclone	4.762	2675736	100.00	0.228	1.377	No

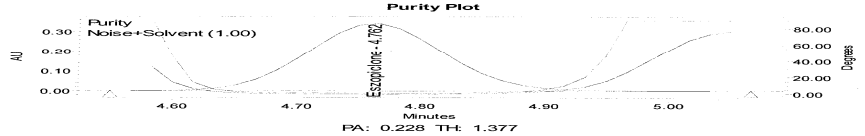
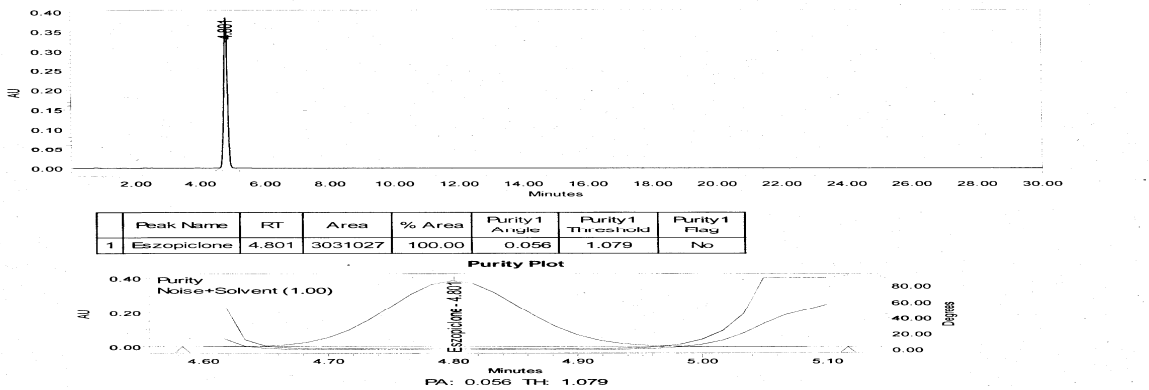
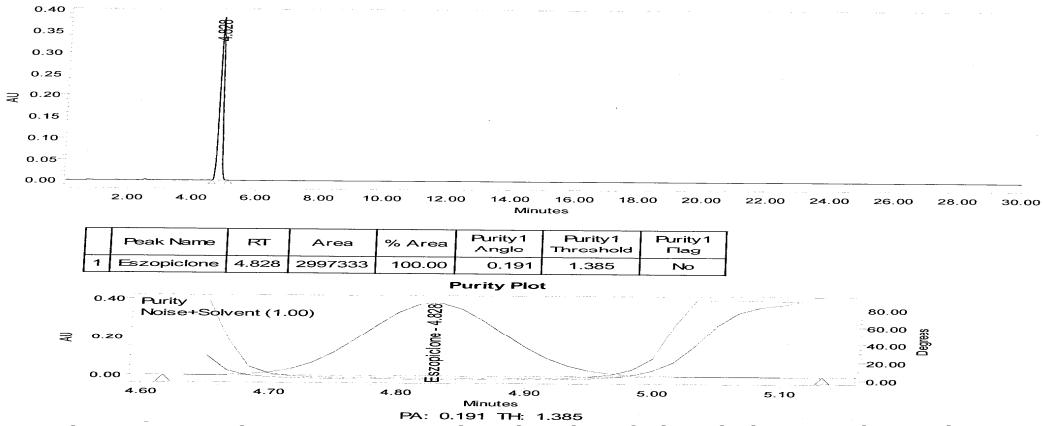


Fig. 7: HPLC Chromatogram and purity plot of Peroxide treated sample



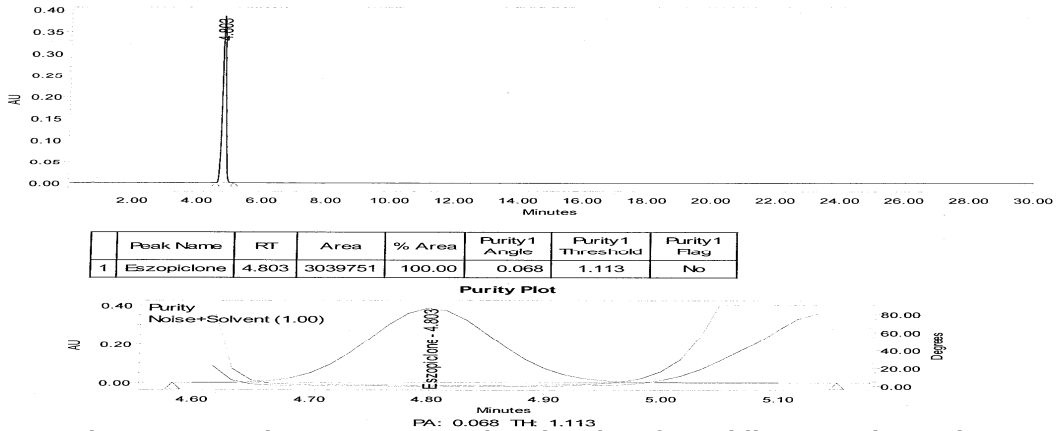
	Peak Name	RT	Area	% Area	Purity1 Angle	Purity1 Threshold	Purity1 Flag
1	Eszopiclone	4.801	3031027	100.00	0.056	1.079	No

Fig. 8: HPLC Chromatogram and purity plot of Thermal treated sample



	Peak Name	RT	Area	% Area	Purity1 Angle	Purity1 Threshold	Purity1 Flag
1	Eszopiclone	4.828	2997333	100.00	0.191	1.385	No

Fig. 9: HPLC Chromatogram and purity plot of Photolytic treated sample



	Peak Name	RT	Area	% Area	Purity1 Angle	Purity1 Threshold	Purity1 Flag
1	Eszopiclone	4.803	3039751	100.00	0.068	1.113	No

Fig 10: HPLC Chromatogram and purity plot of Humidity treated sample

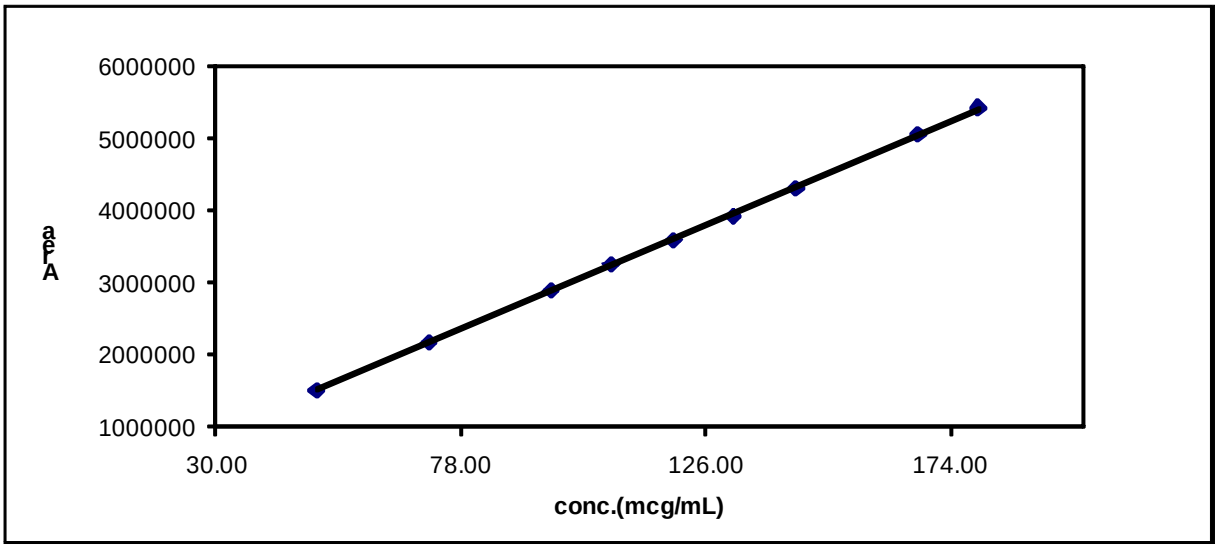


Fig. 11: Linearity plot for Eszopiclone

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