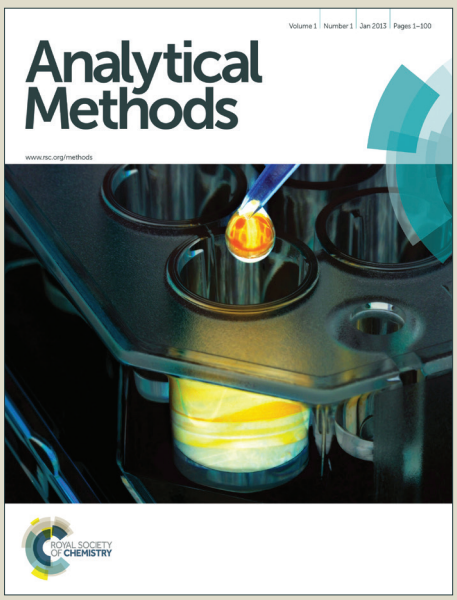
Analytical Methods

Accepted Manuscript



ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/methods

Eco-friendly HPTLC method for assay of Eszopiclone in pharmaceutical preparation: Investigation of its water-induced degradation kinetics

Amira F. El-Yazbi, Rasha M. Youssef*

Faculty of Pharmacy, Department of Pharmaceutical Analytical Chemistry, University of Alexandria, El-Messalah, Alexandria 21521, Egypt

*Tel.: +2034871317; fax: +20 3 4873273. rmmy1973@yahoo.com

Abstract

An eco-friendly, sensitive and selective high-performance thin-layer chromatographic method was developed and validated for analysis of eszopiclone in bulk powder and formulation. The method employed HPTLC aluminium plates precoated with silica gel 60F-254 as the stationary phase. The solvent system consisted methanol–water (6: 4, v/v). Densitometric analysis of eszopiclone was carried out at 300 nm. This system was found to give compact spot for eszopiclone (R_f value of 0.48 ± 0.02). The proposed method was validated for linearity, precision, robustness, LOD, LOQ, specificity and accuracy in accordance with ICH guidelines. Linearity was found to be in the range of 0.2–1.2 µg/band with significantly high value of correlation coefficient r = 0.9997. Statistical comparison with a reported reference method showed similar results with respect to accuracy and precision

Study of the eszopiclone water-induced degradation kinetic was carried out by the proposed HPTLC method. Activation energy and degradation rate constant at room temperature were calculated after construction of Arrhenius plot.

Keywords: Eszopiclone; HPTLC; Tablets; Water-induced degradation kinetics; Arrhenius plot

1. Introduction

Eszopiclone (EZP), (*S*)-6-(5-Chloro-2-pyridinyl)-7-oxo-6, 7-dihydro-5*H*- pyrrolo[3,4*b*] pyrazin5-yl-4-methyl-1-piperazinecarboxylate (Fig. 1), is a short acting nonbenzodiazepine sedative hypnotic used for treatment for insomnia. EZP is the active dextrorotatory stereoisomer of zopiclone, and belongs to the class of drugs known as cyclopyrrolones. EZP has fewer anticholinergic side effects than racemic zopiclone [1].

Being a recently developed drug, only few analytical methods have been reported for EZP determination. These methods include spectrophotmetric [2, 3], HPLC [4-7], UHPLC [8] and TLC [9, 10].

HPTLC is becoming a routine analytical technique [11-13] due to its advantages of low operating cost and need for minimum sample clean up. The major advantage of HPTLC is that several samples can be run simultaneously using a small quantity of mobile phase.

In previous methods of EZP analysis, lots of organic solvents were used which are poisonous, and causing atmosphere contamination. So using methanol and water as mobile phase in HPTLC technique is a proper choice to preclude the use of organic solvents.

Therefore, the objective of this work was to develop eco-friendly, novel and validated HPTLC method for determination of EZP in pharmaceutical preparation. Water induced-degradation kinetics was investigated by the proposed validated method. Activation energy and degradation rate constant at room temperature were calculated after construction of Arrhenius plot.

2. Experimental

2.1. Materials and Reagents

Pharmaceutical grade of EZP was kindly supplied as a gift sample by Medizem Pharmaceutica Industries, Borg El Arab industrial 4th Zone Block 2, Alexandria, Egypt. The pharmaceutical

preparation was purchased from a local pharmacy. The commercial NightCalm® tablets formulation (labeled to contain 3 mg EZP per tablet, batch no. 12427) is purchased from a local pharmacy. It is manufactured by Medizem Pharmaceutica Industries, Borg El Arab industrial 4th Zone Block 2, Alexandria, Egypt. All chemicals and reagents used were of analytical grade and were purchased from Merck Chemicals, Egypt.

2.2. Instrumentation

The samples were applied to the plates using a 100-µL CAMAG microsyringe (Hamilton, Bonaduz, Switzerland) in the form of bands using a CAMAG Linomat IV (Switzerland) applicator. Slit dimension was kept at 6 × 0.2 mm and a 20-mm s^{-1} scanning speed was employed. Ascending development of the mobile phase was carried out in 20 × 20 cm twin trough glass chamber (CAMAG, Muttenz, Switzerland) and the top of chamber was covered tightly with the lid. The optimized chamber saturation time for mobile phase was 30 min at room temperature (25 °C ± 2). CAMAG TLC Scanner III operated in the reflectance–absorbance mode and controlled by CATS software (V 3.15, CAMAG). The source of radiation utilized was deuterium lamp emitting a continuous ultraviolet (UV) spectrum between 190 and 400 nm.

2.3.Chromatographic conditions

Chromatographic studies were performed using the following conditions:

Stationary phase: Silica gel 60 F pre coated HPTLC 254 plates (20 × 10 cm, aluminum plates with 250-µm thickness), Prewashing with methanol, drying in oven (60±1ºC, 5 min),

Mobile phase: Methanol-water (6: 4, v/v)

Volume of mobile phase: 20 ml,

Chamber saturation time: 30 min,

Temperature: 25±1ºC

Migration distance: 80 mm

Band width: 5 mm, space between two bands: 4mm

Wavelength of detection: 300 nm

2.4. Construction of calibration curve

Stock standard solution of EZP was prepared in water at 200 µg/ml. working solutions were prepared by dilution of the stock solution with methanol to give solutions containing EZP in concentration range of 10–60 µg/ml. Twenty microlitre from each working solution was spotted on the HPTLC plate as bands to obtain final concentration range of 0.2–1.2 µg/band. Triplicate applications were made for each solution. The plate was then developed as described in the chromatographic conditions section. The peak areas at R_f = 0.48±0.02 were measured. A calibration graph was constructed by plotting peak areas versus concentrations of EZP to obtain a linear relationship (Table 1).

2.5. Analysis of the marketed formulation

Twenty tablets of NightCalm® (batch no. 12427) were weighed and finely powdered. A portion of the tablet powder equivalent to about 50 mg EZP was weighed and accurately transferred into other 25-mL volumetric flask using about 10 mL methanol. The sample solution was sonicated for 30 min. Dilution was made to volume with methanol followed by centrifugation at 4000 rpm for 5 min. Dilution was made with water to obtain 200 µg/ml sample solution. The general procedure described under construction of calibration graphs was followed and the concentration EZP was calculated.

2.6. Neutral hydrolysis

50 ml of stock solution was refluxed for 1.0 h at 90 °C to study the degradation under neutral conditions. 1.25 mL was quantitatively transferred to 5 mL-volumetric flask using microsyringe

and volume of flask was completed to mark with methanol. Twenty microlitre of this resultant solution was applied on HPTLC plate and the chromatogram was run as described in Section 2.3.

2.7. Study of water-induced degradation kinetics

Accurately weighed 200 mg of drug were dissolved in 100 ml methanol. Separate 5 ml aliquots of this standard solution were transferred into separate 100 ml of double neck round bottom flask and mixed respectively with 45 ml double distilled water to get final concentration of 200 µg/ ml. The flasks were refluxed at different temperatures (40, 70, 80, 90 and 100 °C) for neutral degradation for different time intervals. At specified time intervals, the volumes of 1.25 mL were quantitatively transferred to 5 mL-volumetric flasks with the help of micro syringe and volumes were completed to the mark with methanol. Then 20 µl were spotted to get the final concentration of 1 µg/band. The experiment was carried out in triplicate. The concentration of the remaining drug was calculated for each temperature and time interval. Data were further processed and degradation kinetics constants were calculated.

3. Results and discussion

3.1. Optimization of chromatographic conditions

The experimental conditions, such as mobile phase composition and wavelength of detection were optimized to provide accurate, precise and reproducible compact flat bands for the determination of EZP. Different eco-friendly solvent systems were tried. Finally, the mobile phase consisting of methanol–water (6: 4, v/v) gave a sharp and symmetrical peak. A scanning wavelength of 300 nm was chosen as a suitable wavelength for determining EZP without interference from other excipients in its pharmaceutical formulation. Well-defined bands were obtained when the chamber was saturated with the mobile phase for 30 min at room temperature (Fig. 2). It was required to eliminate the edge effect and to avoid unequal

solvent evaporation losses from the developing plate that can lead to various types of random behavior usually resulting in generally lack of reproducibility in R_f values.

3.2. Validation

International Conference on Harmonization (ICH) guidelines [14] were followed for validation of the developed HPTLC method.

3.2.1. Verification of Accuracy and Precision

In order to evaluate the accuracy and precision of the proposed methods, at least five replicate determinations at three concentration levels were carried out. The concentrations of the drug were within its linearity range. The assay was repeated five times on the same day for studying the intra-day precision (repeatability) and on five different days for studying the inter-day precision for each concentration. The analytical results obtained from this investigation are summarized in table 2.

The low values of the percentage relative standard deviation (% RSD) and the percentage relative error (% Er) (less than 2%) indicate the high precision and good accuracy of the proposed methods.

3.2.2. Verification of Linearity and Range

The linearity of the method was evaluated by analyzing series of different concentrations of EZP. According to ICH, at least five concentrations must be used. Under the experimental conditions described, the graphs obtained by plotting peak area versus concentration (in the ranges stated in table 1) show linear relationships. Statistical parameters and linearity results [15] are depicted in table 1. For equal degrees of freedom, increase in the variance ratio (F-values) means increase in the mean of squares due to regression and decrease in the mean of squares due to residuals. The greater the mean of squares due to regression, the more the steepness of the regression line is.

The smaller the mean of squares due to residuals, the less the scatter of the experimental points around the regression lines. Consequently, regression lines with high F-values (low significance F) are much better than those with lower ones. Good regression lines show high values for both (r) and (F) values [16].

3.2.3. Verification of Limit of Detection (LOD) and Limit of Quantitation (LOQ)

Limit of detection (LOD) is considered as the concentration which has a signal-to noise ratio of 3:1. For limit of quantitation (LOQ), the ratio considered was 10:1 with a RSD% value less than 10% [15]. Using the proposed method, LOD and LOQ were calculated and are presented in table 1.

3.2.4. Robustness

The robustness of the both methods was evaluated by analyzing EZP at the three concentration levels 0.2, 0.8 and 1.2µg band^{-1}. The parameters studied for the HPTLC method were mobile phase composition, mobile phase volume, duration of saturation and time from chromatography to scanning (Table 3). It was found that small variations in the previously-mentioned parameters had no significant influence on the determination of EZP using the proposed method. The low values of RSD% obtained after introducing small deliberate changes in those parameters indicated the robustness of the developed method.

3.2.5. Specificity

The specificity of the HPTLC method was ascertained by analyzing standard drugs and samples. The spot for EZP in samples was confirmed by comparing the R_f and spectra of the spot with those of standards. The peak purity of OST was assessed by comparing the spectra at three different levels, i.e. peak start (S), peak apex (M) and peak end (E) positions of the spots of both drugs. The calculated r (S, M) and r (M, E) values were 0.9999 and 0.9996, respectively. These values indicated the homogeneity of the peaks. In addition, good correlation (r=0.9998) was also

obtained between standard and sample spectra of EZP which is indicated by the superimposed UV spectra of EZP in samples and standard (Fig. 3).

3.3. Spot stability

The time the sample is left to stand on the solvent prior to chromatographic development can influence the stability of separated spots and is required to be investigated for validation [17]. Two-dimensional chromatography using same solvent system was used to find out any decomposition occurring during spotting and development. In case, if decomposition occurs during development, peak (s) of decomposition product (s) shall be obtained for the analyte both in the first and second direction of the run. No decomposition was observed during spotting and development.

3.4. Analysis of the marketed formulation

The proposed HPTLC method was applied to the determination of EZP in its commercial tablets. Satisfactory results were obtained and were in good agreement with the label claims (Table 5). Excellent percentage recoveries and SD suggested that there is no interference from excipients which are present in NightCalm® tablets as hypermellose (methocel E15), PEG 6000, titanium dioxide, purified talc, brilliant blue lake, lactose monohydrate, rose caramellose Na, microcrystalline cellulose, colloidal silicon hydroxide, magnesium stearate and povidone K30. Statistical analysis of the results obtained by the proposed method and reported method [6] was performed using the Student's t-test and the variance ratio F-test (Table 4). The calculated values did not exceed the theoretical ones, indicating no significant difference in the performance of the compared methods regarding accuracy and precision.

3.5. Neutral hydrolysis

The HPTLC chromatogram for neutral degradation showed decrease in peak area of standard till disappearance of peak at end of neutral degradation course without corresponding rise in new peak (Fig. 4). Thus indicates the conversion of standard EZP to non-chromophoric compound.

3.6. Degradation kinetics

According to Garrett and Carper method [18], the k values (reaction rate constant for the decomposition of a drug in solution at various elevated temperatures) are obtained from the linear expression in the following equation:

$\log C = \log C_o - k\,t/2.303$ ----------(1)

where the slope of the line is - k/2.303, C_t and C_o are the concentrations of intact EZP at a given time t and at zero time, respectively.

The logs of the rates of decomposition are then plotted versus the reciprocals of the absolute temperature [19]. Under the previously described experimental conditions, the kinetic of degradation of EZP was investigated. Experiment was repeated three times at each temperature and time interval. The mean concentration of EZP was calculated. A regular decrease in the concentration of EZP with increasing time intervals was observed. At the selected temperatures (40, 70, 80, 90 and 100 °C for water-induced degradation), the degradation process followed pseudo first-order kinetic (Figs. 5). From the slopes of the straight lines, it was possible to calculate apparent first degradation rate constant, half life ($t_{1/2}$) and t_{90} (time where 90% of original concentration of the drug is left) at each temperature for water-induced degradation processes determined by HPTLC method (Table 5). Data obtained from first-order kinetics treatment were further subjected to fitting in Arrhenius equation:

$$\text{Log } K = \text{Log } A - \frac{Ea}{2.303RT} \quad \text{----------(2)}$$

Where K is rate constant calculated from the slope of first order plots, A is frequency factor, Ea is energy of activation (Cal mol $^{-1}$), R is gas constant (1.987 cal/deg mol) and T is absolute temperature (^{o} K). A plot of (4 + log k_{obs}) values versus ($1/T\times10^{3}$) was obtained (Fig. 6), Arrhenius plot was found to be linear in the temperature range of 40–100 °C. The activation energy was calculated for aqueous degradation processes determined by HPTLC method. The degradation rate constant at room temperature ($k_{25}{}^{o}{}_{C}$) is obtained by extrapolating to 25 °C (where 1000/T = 3.356) by inserting this into Eq. 2. The summary of water-induced degradation kinetics data for EZP at 25±2◦C were calculated and presented in table 6.

The $k_{25}{}^{o}{}_{C}$ obtained has the value of 0.003h^{-1}. The time required for the drug concentration to fall to 95, 90, 80% ...etc, of original value is calculated from the equation:

$$t = 2.303/\ k25^{o}C \quad \log C_o/C \quad \text{----------(3)}$$

According to Eq. 3, it was found that 5% of EZP aqueous solution will degrade within 17.10 h or 1% within 3.35 h ($t_{1/2}$, half life time, = 231 h). Finally it could be concluded that aqueous solution of EZP can be used for the analytical purpose within the few working hours at room temperature. Longer life-time is expected if the solution is kept in refrigerator at 8 °C. So, it is advised to keep aqueous stock solution in the refrigerator over the day and on time the working solutions, needed to be used, are prepared by suitable dilution.

4. Conclusion

Introducing HPTLC in pharmaceutical analysis represents a major step in terms of quality assurance. The developed HPTLC technique is eco-friendly, precise, specific and accurate.

Statistical analysis proves that the method is suitable for the analysis of eszopiclone as bulk drug and in pharmaceutical formulations without any interference from the excipients. Comparing with previous published papers using HPLC or UHPLC for eszopiclone determination, proposed HPTLC method has its advantages of low operating costs, using eco-friendly mobile phase, and the need for minimum sample preparation. The major advantage of HPTLC is that several samples can be run simultaneously using a small quantity of mobile phase. The proposed HPTLC method has proved to be more selective and specific than previous published spectrophotometric method.

The proposed HPTLC method is better than the previously described UPLC method for separation of eszopiclone impurities [8] as it studied the degradation kinetic of eszopiclone without using organic solvents that may cause atmospheric contamination. Also the degradation rate constant, half-life and t_{90} of eszopiclone can be predicted.

Acknowledgement

The authors are thankful to Prof. Dr. Fawzy A. Elyazbi, Professor of pharmaceutical analytical chemistry, faculty of pharmacy, university of Alexandria, for providing facilities and gift sample of standard Eszopiclone to carry out this research work. Authors also thank Medizem Pharmaceutica Industries, Borg El Arab industrial 4th Zone Block 2, Alexandria, Egypt, for supplying pharmaceutical grade of Eszopiclone.

References

1 P.A. Patil and M.A. Kothekar, Indi. J Med Sci, 2006, 60 (10), 427–37.

2 K. Anandakumar, G. Kumaraswamy, T. Ayyappan, A.S.K. Sankar and D. Nagavalli, Res. J. Pharm.Tech, 2010, 3, 202.

3 M. Chennaiah, T. Veeraiah and G. Venkateahwarlu, J. Chil. Chem. Soc, 2012, 57, 1460-

1463

4 M. Meng, L. Rohde, V. Capka and P. Bennett, Tandem Labs, 2007, 1-14

5 R.N. Kumar, G.N. Rao, P.Y. Naidu, Asian J. Res. Chem, 2010, 3, 374

6 S.R. Dhaneshwar, V. K. Bhusari, Int. J. ChemTech. Res, 2011, 3, 680

7 K. Shaikh, A. Patil and S. Gite, J. chrom. Sci.,2014, 52(4), 293-297

8 N. Sharma, S. Rao, N. Kumar and A. M. Reddy, The Journal of AOAC Int., 2013, 96, 981 - 986

9 N. Salama, H. Zaazaa, L. Abd El Halim and L. Abd El Fatt, J. plan. Chrom. Mod. TLC, 2014, 27, 166-173

10 B. Paw and G. Misztal, J. plan. Chrom. Mod. TLC, 2000, 13, 195-198

11 H. M. Maher and R.M. Youssef, Chromatographia, 2009,69**,** 345–350

12 R. M. Youssef, H. M. Maher, E. I. El-Kimary, E. M. Hassan and M. A. Barary, The Journal of AOAC Int., 2013, 96 (2) **,** 313-323

13 E.I. El-Kimary, R.M. Youssef and A. N. Allam, J. plan. Chrom. Mod. TLC, 2014, 27, 377–384

14 ICH Guidelines Q2A, Q2A (R1), Validation of Analytical Procedures: Text and Methodology, Geneva, Switzerland, 2005

15 J. N. Miller and J. C. Miller, Statistics and Chemometrics for Analytical Chemistry, Prentice Hall/Pearson, 2010

16 P. Armitage and G. Berry, Statistical Methods in Medical Research, Blackwell Scientific Publications, Oxford, England, 3rd edn, 1994

17 P.D. Sethi, High Performance Thin Layer Chromatography, Quantitative Analysis of Pharmaceutical formulations, CBS Publishers, NewDelhi, 1996

18 E.R. Garrett, R.F. Carper, J. Am. Pharm. Assoc., 1955, 44, 515

19 **A.** Martin, J. Swarbrick and A. Cammarata, Physical pharmacy: Physical chemical principles in the pharmaceutical sciences, 3rd Ed., Lea and Febiger, Philadelphia, PA, 1983

Table 1: Regression and statistical parameters for the determination of eszopiclone using the proposed HPTLC method.

Parameters	Proposed HPTC method
Linearity range (µg band^{-1})	0.20-1.20
LOQ (µg band^{-1})	0.20
LOD (µg band^{-1})	0.06
Intercept (a)	1226.60
Slope (b)	4186.00
Correlation coefficient (r)	0.9964
S_a	135.29
S_b	145.88
$S_{y/x}$	65.24
F	823.35
Significance F	1.21×10^{-3}

S_a is standard deviation of intercept, S_b is standard deviation of slope and $S_{y/x}$ is standard deviation of residuals

Table 2: Evaluation of accuracy and precision of the proposed HPTLC method for the determination of eszopiclone.

Concentration[a]	Mean % Found ± SD[b]	RSD %[c]	Er %[d]
(a) Accuracy and intra-day precision (repeatability)			
0.20	100.10 ± 1.54	1.54	0.10
0.60	101.34 ± 1.90	1.89	1.34
1.20	100.50 ± 1.24	1.23	0.50
Mean	**100.65 ± 1.56**	**1.55**	**0.65**
(b) Accuracy and inter-day precision			
0.20	100.20 ± 1.86	1.86	0.20
0.60	101.55 ± 1.40	1.38	1.55
1.20	101.21 ± 1.26	1.25	1.21
Mean	**100.99 ± 1.51**	**1.50**	**0.99**

a. Final concentration in μg band^{-1}
b. Mean ± standard deviation of five determinations.
c. Percentage relative standard deviation.
d. Percentage relative error.

Table 3: Robustness of the proposed HPTLC method for the determination of eszopiclone.

Parameters	**Mean % Found of peak areas ± RSD[a]**	**R_F ± SD**
1) Mobile phase composition (Methanol–water [5.9:4.1, 6.0:4.0, and 6.1:3.9 *v/v*])	100.90±2.78	0.46±0.015
2) Mobile phase volume (, 20±5mL)	99.76±0.39	0.47±0.009
3) Duration of saturation (30±10min)	100.49±0.58	0.48±0.005
4) Time from chromatography to scan (35±25 min)	99.24±0.44	0.47±0.001

a. Mean ± Percentage relative standard deviation of average of three concentrations; 0.2, 0.8, and 1.2 μg band^{-1}

Table 4 : Statistical comparison for the determination of eszopiclone in its tablets by the proposed method and that of reported one

Preparation	Mean found± RSD% [a]	
	Proposed HPTLC method	Reported method [b]
NightCalm® tablets (3 mg EZP per tablet) batch no. 12427	99.05±0.79	98.71±1.72
t[c]	0.402	
F[c]	4.74	

a. Percentage relative standard deviation for five determinations, Percentage recovery from the label claim amount.
b. HPLC method (6).
c. Theoretical values of t and F for p=0.05 are 2.31 and 6.39, respectively.

Table 5: Degradation rate constant (K_{obs}), half-life ($t_{1/2}$) and t_{90} for eszopiclone in neutral medium determined by proposed HPTLC method

Temperature (°C)	K_{obs} (h^{-1})	$t_{1/2}$(h)	t_{90}(h)
40	0.019	36.4737	5.5463
70	0.4168	1.6625	0.2528
80	1.4870	0.4660	0.0709
90	3.4953	0.1983	0.0301
100	6.2236	0.11135	0.0169

Table 6: Summary of degradation kinetic data of eszopiclone at 25 °C using proposed HPTLC method

Parameters	data
Ea (kcal mol^{-1})[a]	22.967 X10^{-3}
$K_{25°C}$[b]	0.003
$t_{1/2}$(h)[c]	231
t_{90}(h)[d]	9.625

a. Activation energy.
b. Degradation rate constant.
c. Half-life.
d. Time left for 90% potency

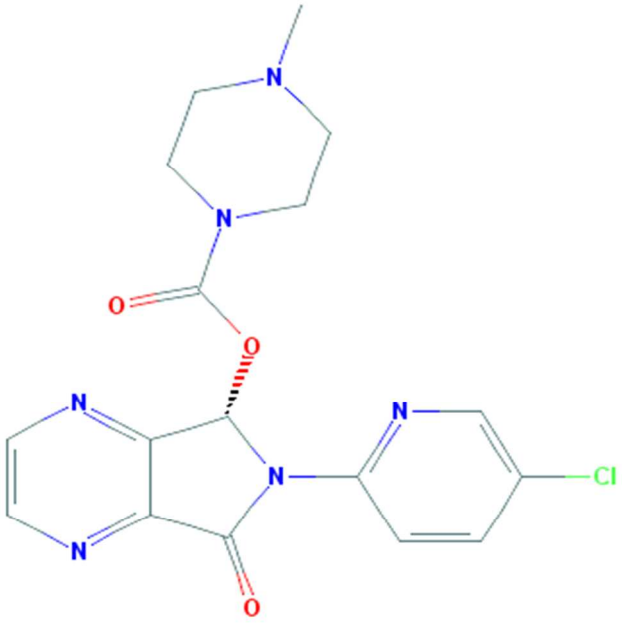


Fig. 1: Chemical structure of eszopiclone

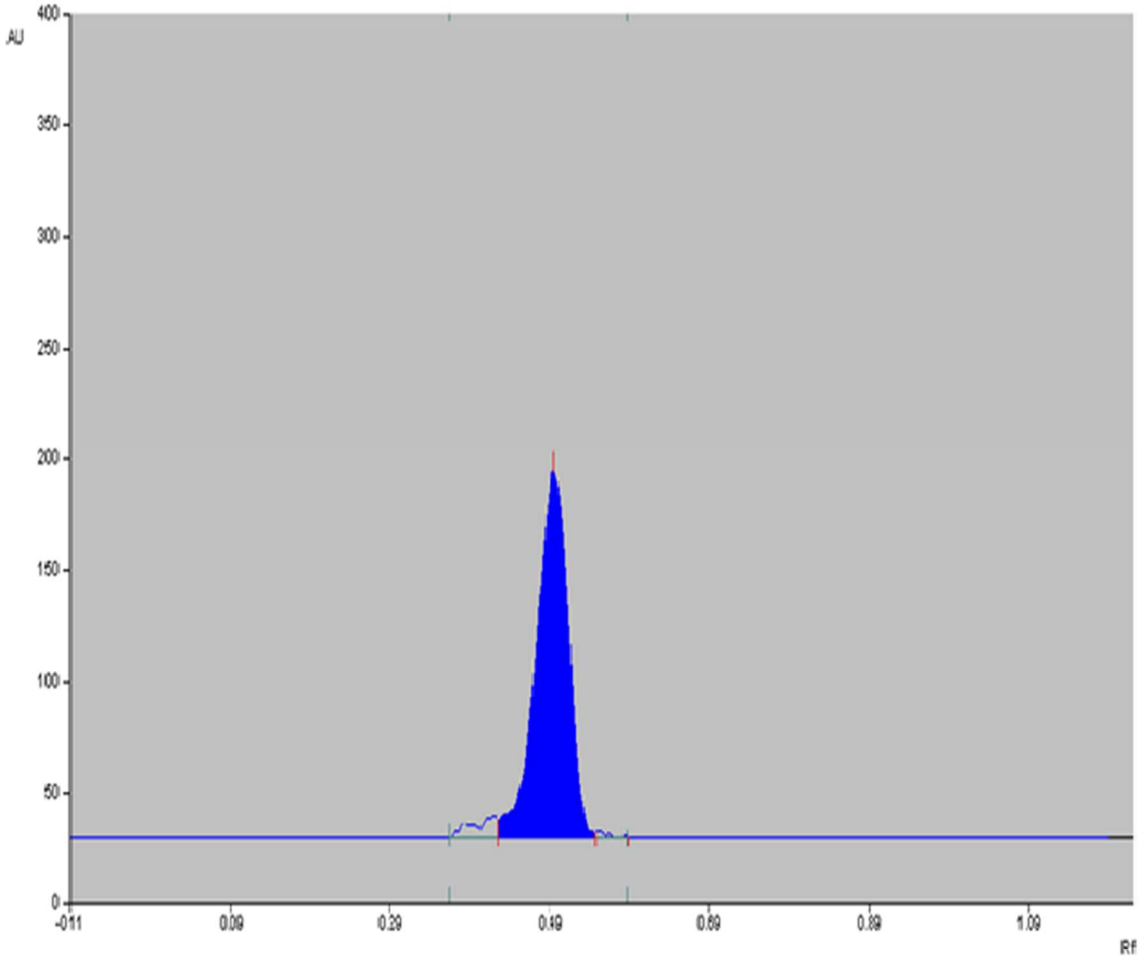


Fig.2: HPTLC chromatogram of standard eszopiclone (1.0 µg band^{-1}); (*R*f: 0.48 ± 0.02), mobile phase of methanol–water (6: 4, v/v)

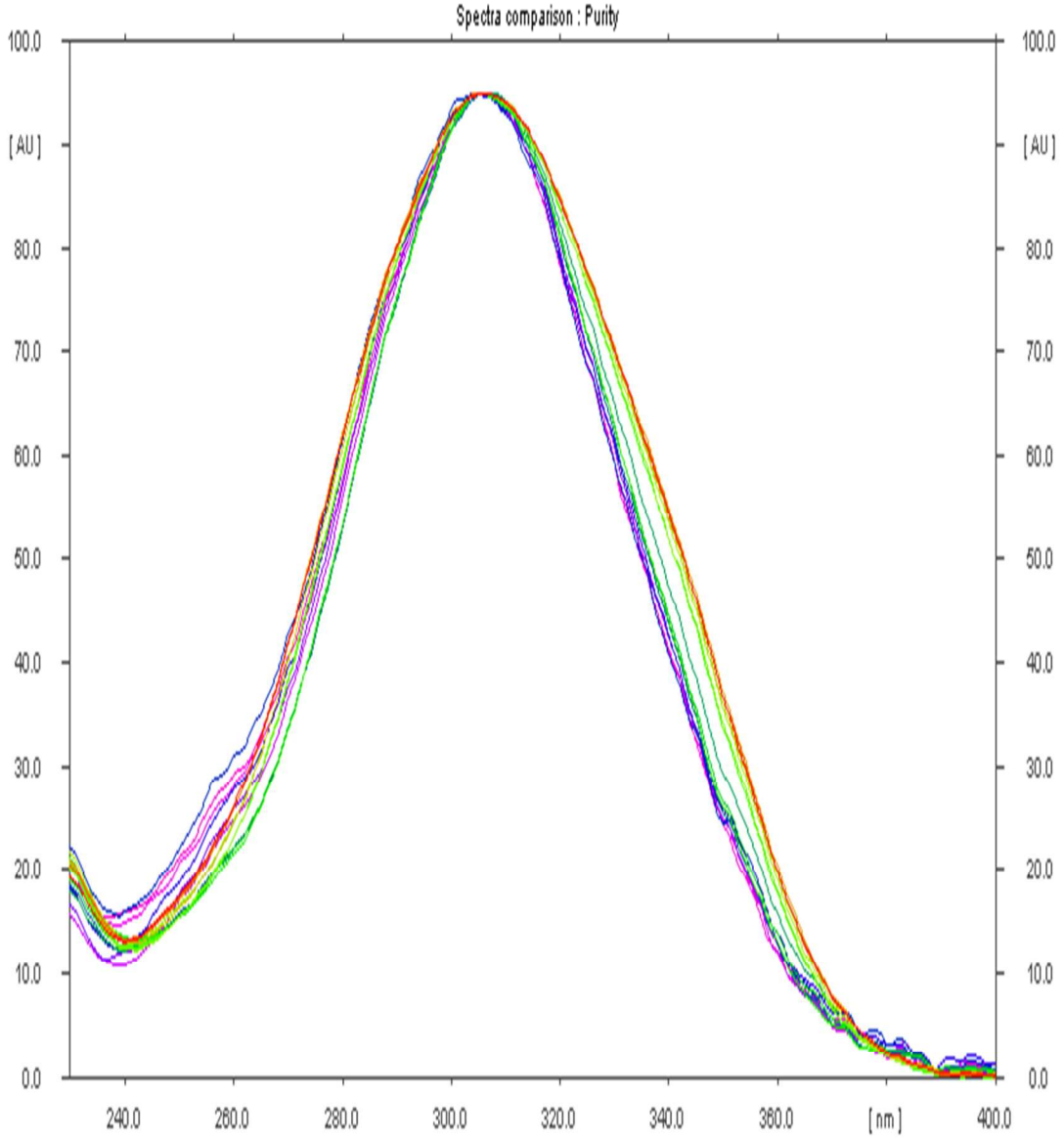


Fig.3: Spectrum illustrating peak purity of Ezopiclone obtained from corresponding standard and tablets

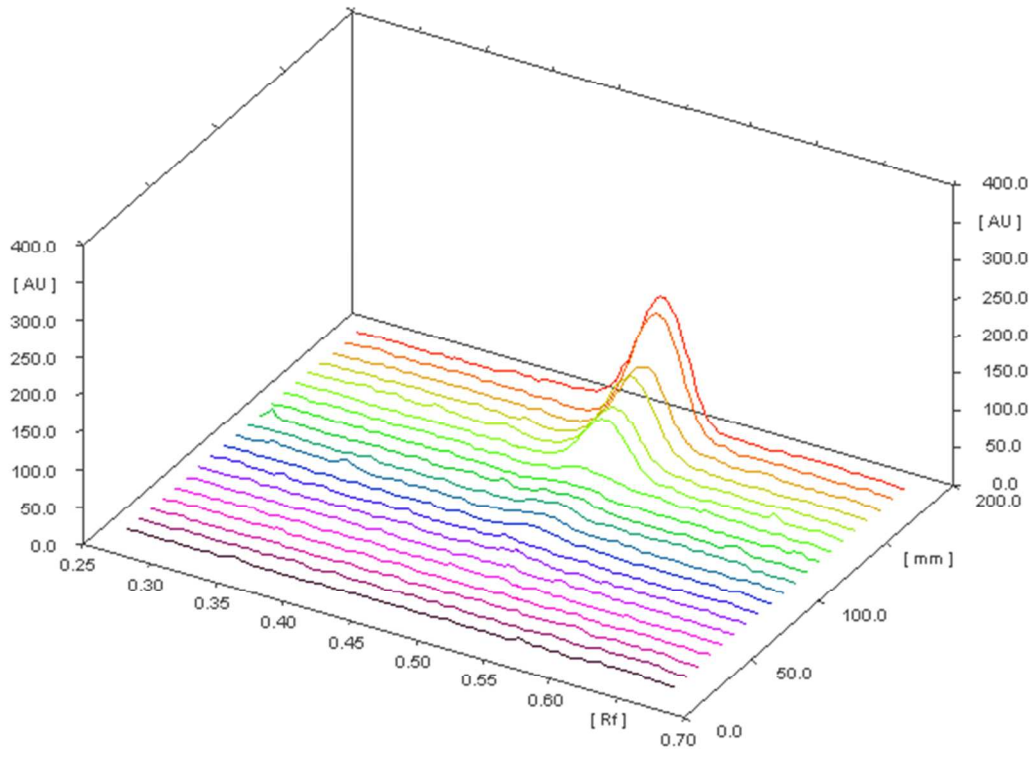


Fig.4: Three-dimensional overlay of HPTLC chromatograms obtained from 1.0 µg band^{-1} eszopiclone refluxing at 90°C in neutral medium for different time intervals; all tracks were scanned at 300 nm

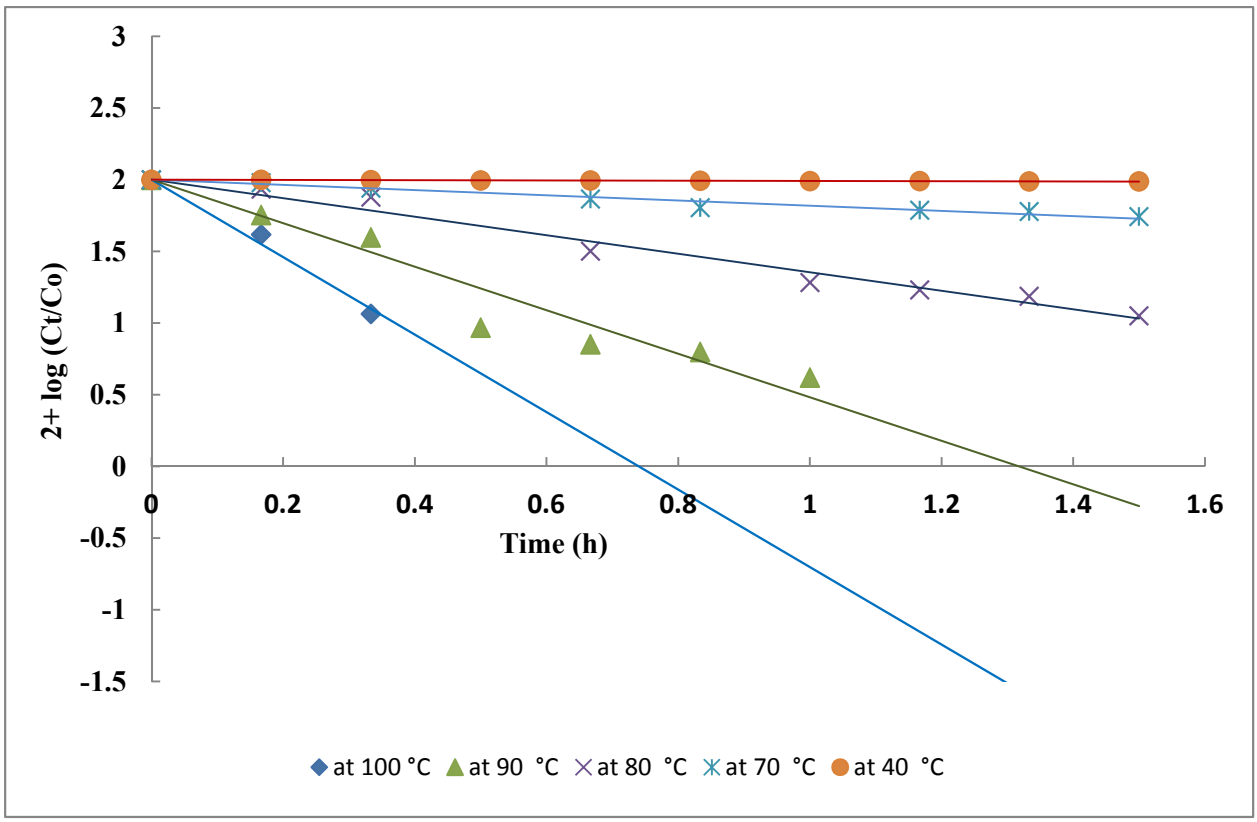


Fig. 5: Pseudo first-order plots for the water-induced degradation of eszopiclone at various temperatures using HPTLC method; C_t, concentration at time t; C_0, concentration at time zero.

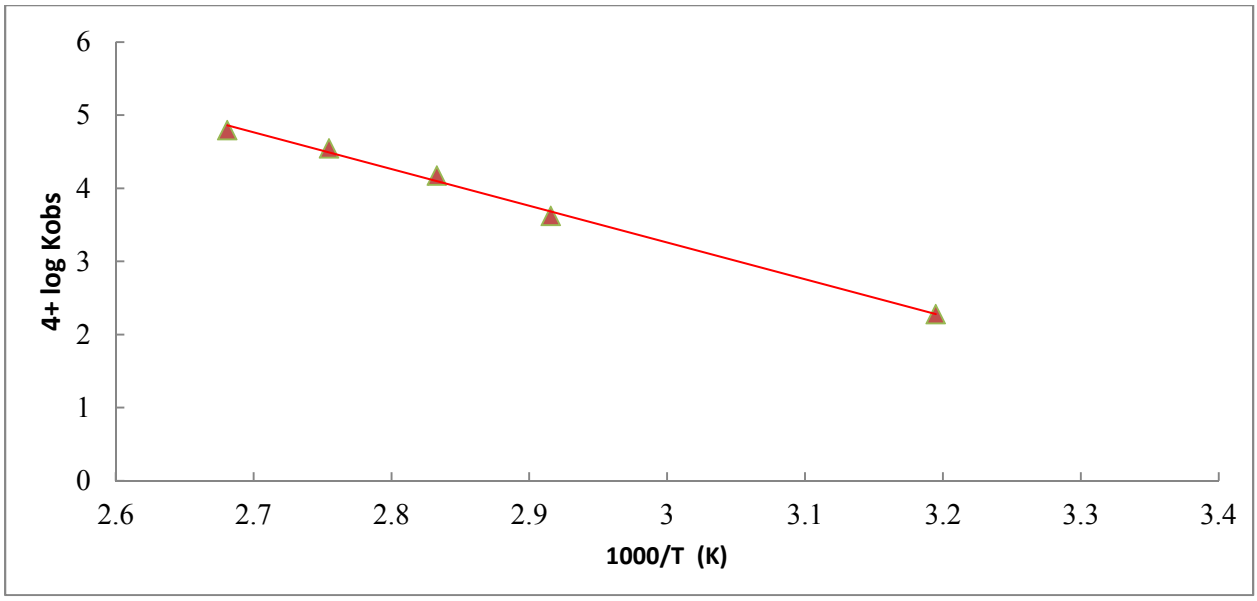
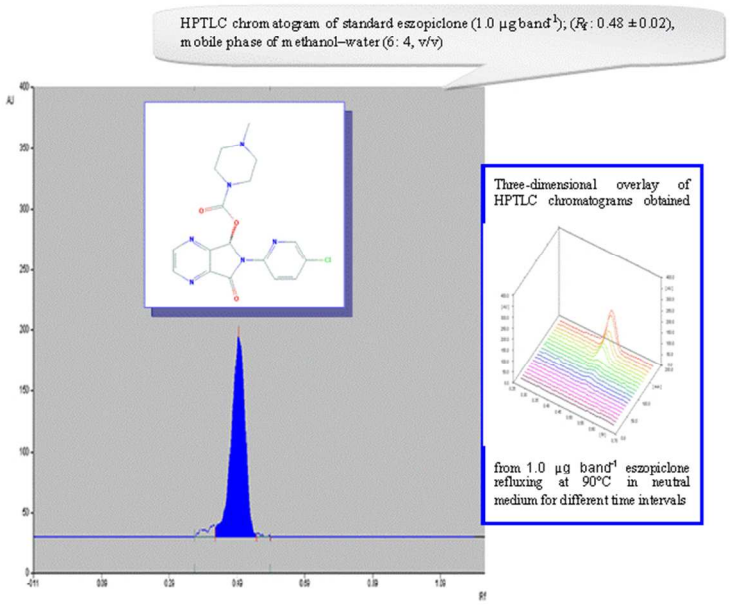


Fig.6: Arrhenius plot for the water-induced degradation of eszopiclone



287x372mm (72 x 72 DPI)