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Original Article

Validated Stability-indicating NP-HPTLC/Densitometry Method for the Assay of Zolpidem Tartrate in Pharmaceutical Dosage Form

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ABSTRACT

Background: A simple, precise stability-indicating Normal- Phase Thin-Layer Chromatography (NP- TLC)/ Densitometry method has been studied for estimation of zolpidem tartrate in bulk and in tablet formulation. **Method:** The chromatographic separation was accomplished on aluminium backed precoated silica gel 60 F_{254}S as the stationary phase using *Ethyl acetate: Methanol: triethylamine (9: 1: 0.3 v/v)* as mobile phase. Densitometric analysis of zolpidem tartrate was achieved at λ max 293 nm. The method was validated for robustness, precision and accuracy. Stress degradation of zolpidem tartrate was carried out under various reaction conditions including acid, base, oxidation, photo-degradation and dry heating treatment. **Result:** This system was found to give compact spot for zolpidem tartrate at Rf value 0.56 ± 0.02. The data of linear regression analysis of zolpidem tartrate indicated a good linear relationship over the range of 300 – 1800. LOD and LOQ found 18.10 and 54.84 ng. Resulted stress drugs were analyzed with the developed TLC/ densitometry method. **Conclusion:** Statistical analysis proves that the method is repeatable and selective for the estimation of zolpidem tartrate in bulk and in formulation. As the method could effectively separate the drug from its degradation products, it can be employed as a stability- indicating method.

Key words: Zolpidem Tartrate, Stability-indicating, Validation, TLC.

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INTRODUCTION

Zolpidem Tartrate (Zolpidem tartrate) N, N-dimethyl-2-[6-methyl-2-(4-methylphenyl) imidazo [1,2-a] pyridin-3-yl] acetamide is a short-acting nonbenzodiazepine hypnotic. Zolpidem is used in the short-term treatment of insomnia[1] Figure 1. A detailed literature survey revealed several analytical methods such as HPLC[2-5] HPLC-MS[6] have been reported in pharmaceutical formulation and biological fluid. UV-Spectrophotometry[7-9] reported.

High- Performance Thin- Layer Chromatography (HPTLC) is one of the routinely used analytical techniques due to its small operational cost; high sample through put and required minimum sample clean up. The major advantage of HPTLC is that several samples can be run simultaneously using little quantity of mobile phase unlike HPLC; thus, lowering analysis time and cost per analysis.[10]

The objective of the present investigation is to develop a new simple, precise and accurate NP-TLC/Densitometry method for analysis of zolpidem tartrate in the bulk material and in tablet formulation. Further, to validate the developed method for accuracy, precision, ruggedness and robustness, sensitivity, specificity and stability study is carried out as per ICH guidelines.

Experimental Instrumentation

The optimized chromatographic conditions are as follows;

- Stationary Phase: Aluminium backed silica gel 60 F_{254} S, (20 cm × 10 cm with 250 µm thickness (E.Merck, Mumbai, India) previously washed with methanol and air-dried.
- Mobile phase: Ethyl acetate:Methanol: triethylamine (9:1: 0.3 v/v)
- Chamber saturation: 20 min
- Activation of plate: 10 min
- Bandwidth: 6mm
- Slit dimension: 6.00 X 0.45mm
- Radiation source: Deuterium lamp
- Detection wavelength: 293 nm
- Distance between bands: 15.4 mm

Materials and Reagents

Zolpidem tartrate (99.5%) was obtained as gift samples from Torrent Pharmaceutical Ltd Ahmadabad (India). Ethyl acetate, Methanol and trimethylamine were used of HPLC grade.

Preparation of Stock Standard and sample solution

Stock standard solution was prepared by dissolving 10 mg of Zolpidem tartrate in 10 mL of methanol which gives concentration of 1000 µg.mL^{-1}

To quantify the content of zolpidem tartrate in tablets, twenty tablets were weighed accurately, average weight determined and ground into fine powder. A quantity of powder equivalent to 10 mg of zolpidem tartrate was transfer in to 100 mL volumetric flask containing 30 mL methanol, shaken manually for 30 min and diluted to mark with same solvent. The resulting solution was filtered using 0.45 µm filter (Millifilter, Milford, MA, USA) and an appropriate volume 6 µL equivalent to 600 ng zolpidem tartrate was applied on TLC plate, developed and scanned as described above. The content of drug estimated using linearity curve.

Study of Linearity Curve

The linearity study verifies and approves that the sample solutions are in a concentration range where analyte response is linearly proportional to the concentration. From the stock standard solutions, a proper volume in the range of 0.3 – 1.8 mL was transferred into series of 10 mL volumetric

flask. A fixed volume 10 µL was applied on the TLC plates to obtain concentration 300, 600, 900, 1200, 1500 and 1800 ng per band of zolpidem tartrate, respectively. Each concentration was applied six times to the plates and developed as described above. Peak area plotted against corresponding amount to obtain the calibration plot.

Method Validation

Precision

The precision is the parameter that expresses the closeness of agreement (degree of scatter) between a series of measurement obtained from multiple analysis of the same homogeneous sample under prescribed condition. To determine system precision, repeatability of sample application and measurement of zolpidem tartrate peak area measured by performing six replicate analysis of same concentration (600 ng per band). Method precision was assessed by measuring intra-day and inter-day variation of results from analysis of zolpidem tartrate at three different concentrations (600, 900 and 1200 ng per band).[11]

Robustness and Ruggedness

The robustness of an analytical procedure is a measure of its capacity to stay unchanged by small, but intentional changes in the method parameters and provides an indication of its reliability during normal day-to-day usage. The robustness of method was established by performing test method by studying different parameters like mobile phase volume, mobile phase composition, relative humidity, development distance, duration of saturation, time from spotting to chromatography and chromatography to spotting were studied and the effects on the results were examined. The ruggedness of the proposed method was evaluated by two different analysts using same environmental and experimental condition. The robustness and ruggedness of the method was assessed at concentration (600 ng per band).

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

To determine the limits of detection and quantification, concentration at the low end of the linear range of the calibration plot were analysed. LOD and LOQ were calculated by use of the equation LOD = 3.3 × *N/B* and LOQ = 10 × *N/B;* where, 'N' is the standard deviation of the peak areas of the drugs (i = 3), taken as a measure of noise, and '*B*' is the slop of the corresponding calibration plot.

Specificity

Specificity of an analytical method is its ability to measure accurately and specifically the concentration of analyte (s) of interest without interference from other API, diluents, mobile phase. The specificity of the method was established by analysis of drug standards and samples and comparing the R_F value and spectrum of zolpidem tartrate band from samples with those of the band from standard. The peak purity of zolpidem tartrate was assessed by comparing spectra at three different positions on the band, i.e. peak-start (S), peak apex (M), and peak-end (E).

Accuracy

Accuracy of a method is defined as the closeness of measured value to the true value for the samples. To study the recovery of the drug at different levels in formulations, pre-analysed samples were spiked with 80 %, 100 %, and 120 % extra zolpidem tartrate standard and re-analysed by the proposed method.

Forced Degradation Study

Acid and base-induced degradation was attempted by separately adding 10 mg of zolpidem tartrate 10 mL each of 0.1 N HCl and 0.1 N NaOH solutions. To exclude the possible degradative effect of light these solutions were kept for 8 h at room temperature in dark. From each one of it, 1 mL solution xwas neutralized and diluted up to 10 mL with methanol. The Final resulting solutions (600 ng per band) were applied to a TLC plate and chromatograms were obtained as described in previous section. For oxidative degradation, 10 mg of zolpidem tartrate added in 10 mL ofhydrogen peroxide solution (3 %, (*v/v)*. To exclude the possible degradation effect, this solution was kept for 8 min in dark. The solution (1 mL) was diluted to 10 ml with methanol and treated as described for acid and base-induced degradation. To assess the neutral degradation, a 10 mg of zolpidem tartrate was added in 10 mL methanol and Light heat degradation was studied by exposing 10 mg of Zolpidem tartrate in 10 mL methanol in sun light for 8 h. From each one of it, 1 ml was diluted to 10 mL with methanol.[12] This solution was applied on plate (6 µL each, i.e 600 ng per band) and chromatogram was obtained as described in previous section. For dry heat study, Zolpidem tartrate 10 mg was stored at 60^0 C for 3 h in oven and transferred to 10 mL volumetric flask containing methanol and volume was made up to the mark. 0.6 µl (600 ng/band) was applied on TLC plate in triplicate and chromatogram were run as described above.

RESULTS AND DISCUSSION

Optimisation of TLC/ Densitometry method

To develop the TLC/Densitometry method for the estimation of Zolpidem tartrate, selection of the mobile phase was carried out on the basis of polarity. Initially, combination of various proportions of solvents *n*- hexane, methanol and ethyl acetate were tried as mobile phase but diffusion of the spots and tailing was observed. To avoid the problem, 0.3 ml of triethylamine was added as modifier. Finally, mobile phase composed of *Ethyl acetate:Methanol: triethylamine (9:1: 0.3 v/v)* was established as observed to well resolved and spot with Rf value 0.56 ± 0.02 when plate was developed and scanned at 293 nm; Figure 2. The chamber was saturated with the mobile phase for 20 min at room temperature and plates were activated at 110 ºC for 5 min to obtain well-defined spots.

Study of calibration curve

Linearity Curve for Zolpidem tartrate was observed in the concentration range of 300 - 1800 ng/band. The correlation coefficient (r) found to be 0.9970. Linearity range was established with five replicate readings of each concentration.

Validation of Method

Precision

Precision of the method was determined as repeatability, and intra-day and inter-day variation. The precision of the method was expressed as a percentage relative standard deviation (RSD [%]). The percentage RSD in the repeatability study was found to be 0.83 %. The percentage RSD in intra-day and inter-day study was found to be 0.57 and 0.83 %, respectively. Since the < 2 % indicates method is précised.

Robustness and Ruggedness

As shown in Table 1, the standard deviation and RSD [%] of peak areas was calculated for each change of condition and % RSD < 2% indicates robustness of the method.

The % RSD of results of analysis by two analysts were calculated and found to be 1.09% and 1.03%, respectively. Since, there was no statistically difference between the assay results obtain by each analyst the method proved to be rugged.

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

The sensitivity of the method was determined as LOD and LOQ, found to be 18.10 ng and 54.84 ng, respectively.

Table 1: Results from assessments of the robustness of the methoda

Condition	RSD [%]
Mobile phase composition (± 0.3 mL)	0.81
Mobile phase volume (± 2 mL)	0.76
Development distance (± 0.5 cm)	0.98
Plate saturation time (± 5 min)	1.02
Relative humidity (± 5%)	1.25
Activation of TLC plates previously developed with methanol and dried at 60° C (±2 min)	1.37
Time from application to chromatography (±10 min)	1.10
Time from chromatography to scanning (±10 min)	1.05

a) Results are averages from six determinations

Table 2: Results of recovery studiesa

Component	Label claim (mg/tablet)	% amount of standard drug added	% drug recovered	% RSD
Zolpidem tartrate		80	100.56	1.28
		100	100.27	1.03
		120	99.92	0.73

a) Results are averages from three determination at each level

Table 3: Summary of validation parameters

Parameters	
Linearity range [ng/band]	300 - 1800
Correlation coefficient	0.9970
% Recovery [n = 9],% RSD	1.01
Ruggedness, % RSD	
Analyst I [n = 6]	1.09
Analyst II [n = 6]	1.03
Precision [% RSD]	
Repeatability [n = 6]	0.83
Intra-day [n = 3]	0.57
Inter-day [n = 3]	0.87
Robustness	Robust
Specificity	Specific

n- Results are averages from six determinations

Table 4: Forced degradation study of Zolpidem tartrate

Sample exposure condition	Duration for exposure (hr)	No. of degradation products (Rf value)	Recovery (%)
0.1N HCl	3	2(0.03,0.29)	85.20
0.1NNaOH	9	2(0.10,0.23)	92.51
H2O2	6	3(0.03,0.25,0.35)	75.19
Heat at 60°C	3	1(0.37)	77.37
Sunlight	8	No degradation	99.97

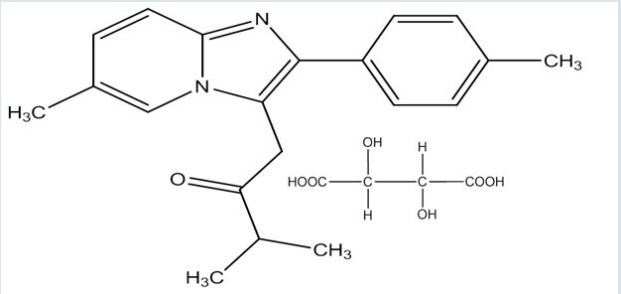

Figure 1: Chemical Structure of zolpidem tartrate.

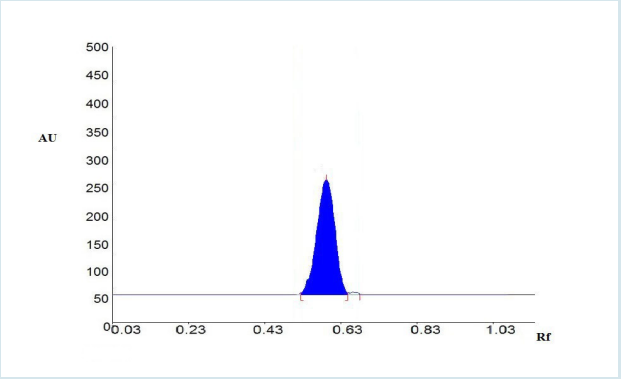

Figure 2: Densitogram of zolpidem tartrate showing Rf value 0.56 at 293 nm.

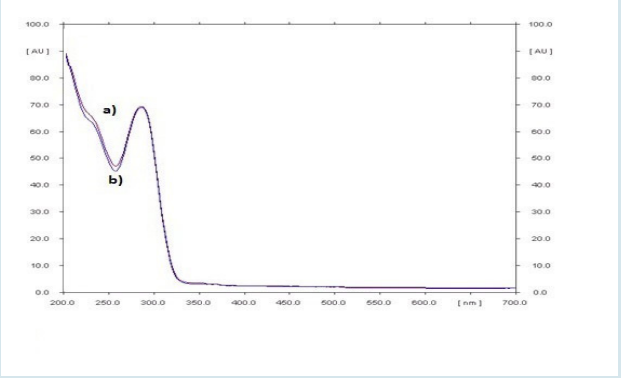

Figure 3: Overlain spectra of Zolpidem tartrate standard [a] and Zolpidem standard extracted from tablet [b] scanned at the peak start, peak apex, and peak end position of band.

Specificity

(Figure 3) showed the results obtained from assessment of the purity of the Zolpidem tartrate peak by comparison of spectra acquired at the peak-start, peak- apex, and peak-end position of the band.

Accuracy

The results obtained from determination of recovery are listed in Table 2; the low % RSD values indicative of the accuracy of the method. Summary of validation Parameter Given in Table 3.

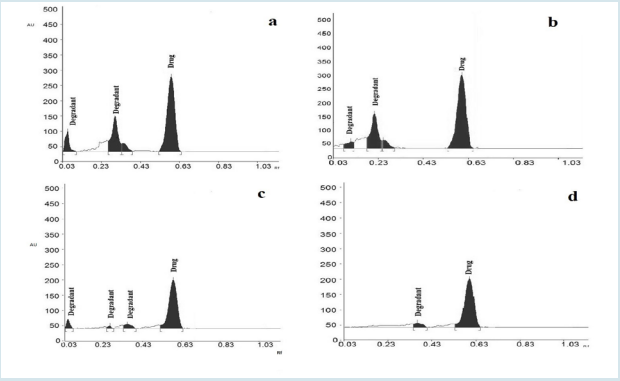

Figure 4: Forced Degradation study on zolpidem tartrate a): Acid degradation, b): Alkali Degradation, c): Oxidative degradation, d) : Dry heat degradation.

Assay of Marketed Formulation

A band at R_f 0.56 for zolpidem tartrate was observed in the densitogram. The drug content was found to be 100.25%, with % RSD 1.01. The low value is indicative of the suitability of method for routine analysis of zolpidem tartrate in pharmaceutical dosage forms

Force Degradation of Zolpidem Tartarate

Forced Degradation study of Zolpidem tartrate carried by action of acid, alkali, hydrogen peroxide, sunlight and dry heat showed that Zolpidem tartrate was degraded in all stressed conditions except sunlight and showed well resolved bands of Zolpidem tartrate and additional peaks at different R_f values i.e. all the degradation product were resolved from Zolpidem tartrate Figure 4. The percentage amount of Zolpidem tartrate remaining and amount of Zolpidem tartrate recovered [%] were calculated (n = 3); these values, and number of degradation products and their R_F values are given in Table 4.

CONCLUSION

The proposed TLC/ Densitometry method provides simple, accurate, stability indicating and reproducible quantitative analysis for estimation of Zolpidem tartrate in tablet formulation. The method was validated as per ICH guidelines.The method is specific and there is no interference from any of the sample components. It can be concluded that the developed method offers several advantages such as rapid, cost effective, simple mobile phase and simple preparation steps, improved sensitivity and comparative short run time.

CONFLICT OF INTEREST

Authors have no conflict of interest

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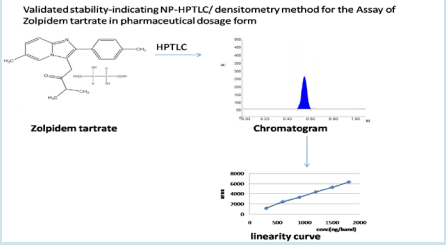
REFERENCES

1. Drug Bank, December 2016, www.drugbank.ca/drugs/DB00425.
2. Mahajan MP, Sawant SD (2012), Stability indicating RP-HPLC method for the estimation of zolpidem tartrate in bulk and tablet dosage form. Int J Pharm Pharm Sci. 4;5:268-74.
3. Guinebault P, Dubruc C, Hermann P, Thénot J. High-performance liquid chromatographic determination of zolpidem, a new sleep inducer, in biological fluids with fluorimetric detection. J. Chromatogr. 1986;1;383:206-11. https://doi.org/10.1016/S0378-4347(00)83462-3.
4. Nirogi RV, Kandikere VN, Shrivasthava W, Mudigonda K. Quantification of zolpidem in human plasma by high-performance liquid chromatography with fluorescence detection. Biomed. Chromatogr. 2006; 20(10):1103-8. https://doi.org/10.1002/bmc.652 ; PMid:16703647.
5. Durol ALB and Greenblatt DJ. Analysis of zolpidem in human plasma by high-performance liquid chromatography with fluorescence detection: application to single-dose pharmacokinetic studies. J. Anal. Toxicol. 1997;21(5):388-92. https://doi.org/10.1093/jat/21.5.388.
6. Bhatt J, Jangid A, Shetty R, Shah B, Kambli, S, Subbaiah G, Singh S. Quantification of zolpidem in human plasma by liquid chromatography–electrospray ionization tandem mass spectrometry.Biomed.Chromatogr. 2006;20(8):736-42. https://doi.org/10.1002/bmc.589 ; PMid:16240286.
7. Mahajan MP and Sawant SD. Validated Spectrophotometric method for the estimation of Zolpidem tartrate in Bulk and Tablet Formulation. Int. J. ChemTech Res. 2012;4(1):403-8.
8. Patil KS, Pore YV, Bhise SB. Spectrophotometric Estimation of Zolpidem in tablets. J. Pharm. Sci. Res, 2010;2(1):1-4.
9. Chomwal R, Kumar A, Goyal A. Spectrophotometric methods for determination of zolpidem tartrate in tablet formulation. J. Pharm Bioallied Sci. 2010;2;(4):365-8. https://doi.org/10.4103/0975-7406.72142 ; PMid:21180474 PMCid:PMC2996077.
10. Shethi PD (1996), HPTLC, Quantitative Analysis of Pharmaceutical Formulation. 1st ed. Delhi: CBS Publisher and Distributors. 1996:1-15.
11. ICH Q2 (R1) (2005), Validations of analytical procedures: Text and Methodology.
12. ICH QA1(R2) (2003), Stability testing of new drug substance and products. International Conference on Harmonization, Geneva.

PICTORIAL ABSTRACT



SUMMARY

Normal phase HPTLC method developed for zolpidem tartrate by usingsolvent system Ethyl acetate:Methanol: triethylamine. The method was validated as per ICH guidelines. The method was specific and there is no interfer-ence from any of the sample components. Forced degradation studies proved that method could effectively separate the drug from its degradation products; it can be employed as a stability- indicating method.

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