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DEVELOPMENT AND VALIDATION OF HPTLC METHOD FOR ESTIMATION OF ZOLPIDEM IN PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

Objective: This paper describes a new, simple, precise, accurate and specific HPTLC method for estimation of zolpidem as a bulk drug and in tablet dosage forms. **Methods:** Chromatographic separation of the drug was performed on aluminum plates pre-coated with silica gel 60 F_{254} as the stationary phase and a mobile phase comprising of water: methanol: acetic acid (8.0: 2.7: 0.3v/v/v). Densitometric quantification of zolpidem was carried out at 245 nm. Zolpidem was detected satisfactorily with an R_f value 0.42±0.03. **Results:** The accuracy and reliability of the method was assessed by evaluation of linearity (100-1000 ng/spot), precision (intra-day RSD 0.52% and inter-day RSD 0.91%), accuracy (99.12-100.10%) and specificity according to ICH guidelines. **Conclusion:** The proposed HPTLC method is simple, precise, accurate, specific, reproducible and less economic without interference from the excipients. The developed method is suitable for routine analysis of zolpidem in bulk and as a tablet dosage form.

KEYWORDS: Zolpidem, HPTLC, Densitometric estimation, Method development, Validation.

INTRODUCTION

Zolpidem chemically is, N,N,6-trimethyl-2-(4-methylphenyl)-imidazo(1,2-a)pyridine-3-acetamide (Fig.1), and is a short-acting nonbenzodiazepine hypnotic that potentiates gamma-aminobutyric acid (GABA), an inhibitory neurotransmitter, by binding to benzodiazepine receptors which are located on the gamma-aminobutyric acid receptors. Its chemical formula

is $(C_{19}H_{21}N_3O)_2,C_4H_6O_6$ with a molecular weight of 764.9 g/mol. It is white hygroscopic crystalline powder slightly soluble in water, practically insoluble in dichloromethane, sparingly soluble in methyl alcohol. Its hypnotic effects are similar to those of the benzodiazepine class of drugs, but it is molecularly distinct from the classical benzodiazepine molecule and is actually classified as an imidazopyridine. Flumazenil, a benzodiazepine receptor antagonist, which is used for benzodiazepine overdose, can also reverse zolpidem's sedative/hypnotic effects. As an anticonvulsant and muscle relaxant, the beneficial effects start to emerge at 10 and 20 times the dose required for sedation, respectively. For that reason, it has never been approved for either muscle relaxation or seizure prevention.[1-2]

Literature survey reveals, selective potentiometric determination of zolpidem hemitartrate in tablets and biological fluids by using polymeric membrane electrodes, determination of zolpidem in human plasma using LC with fluorescence detection, LC-tandem mass spectrometry method for the simultaneous determination of 26 benzodiazepines and metabolites, zolpidem and zopiclone, in blood, urine, and hair, determination and in-process control of zolpidem synthesis by LC, Rapid and simple method for the determination of zolpidem in human plasma by high-performance liquid chromatography and determination of zolpidem hemitartrate by quantitative HPTLC and LC have been reported.[3-7] Zolpidem was determined by HPLC methods together with other drugs, in biological fluids and stability indicating LC, TLC method. To the best of our knowledge no method is reported for determination by HPTLC, hence the primary aim was to develop and validate a HPTLC method for the rapid quantitation of the drug for routine analysis. The present study illustrates development and validation of simple, accurate, economical and reproducible method for determination of zolpidem by HPTLC as bulk and tablet dosage forms as compared to the other methods. The proposed method was validated as per ICH guidelines.

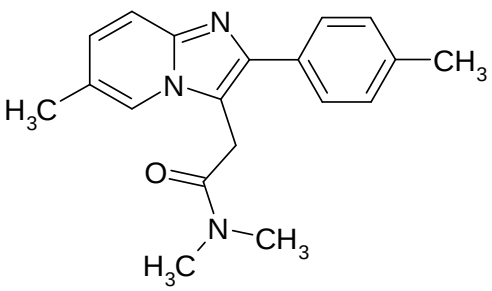

Fig. 1: Structure of zolpidem.

MATERIALS AND METHODS

Reagents and chemicals

Pharmaceutical grade zolpidem was kindly supplied as a gift sample by Shreeji Pharma International, Vadodara, India. It was certified to contain 99.85% (w/w) on dry basis and was used further without purification. All other chemicals and reagents used were analytical grade and were supplied from Merck Chemicals, India.

INSTRUMENTATION

The samples were spotted in the form of bands of width 6 mm with a Camag 100 µl sample (Hamilton, Bonaduz, Switzerland) syringe, on silica gel pre-coated aluminum plate 60 F_{254} plates (10×10 cm) with 250 µm thickness; (E. Merk, Darmstadt, Germany), supplied by Anchrom technologist, Mumbai using a Camag Linomat V (Switzerland) sample applicator . The plates were prewashed with methanol and activated at 110°C for 5 min prior to chromatography. A constant application rate of 0.1 µl/s was used and the space between two bands was 6 mm. The slit dimension was kept at 5 mm × 0.45 mm and the scanning speed was 10 mm/s. The monochromatic band width was set at 20 nm and 320 cut off filter; each track was scanned three times and baseline correction was used. The mobile phase consisted of water: methanol: acetic acid (8.0: 2.7: 0.3; v/v/v) and 11.0 ml of mobile phase was used per chromatography run. Linear ascending development was carried out in a 20 cm × 10 cm twin trough glass chamber (Camag, Muttenz, Switzerland) saturated with the mobile phase. The optimized chamber saturation time for mobile phase was 20 min at room temperature (25°C±2) at relative humidity of 60% ±5. Each chromatogram was developed over a distance of 8 cm. Following the development, the TLC plates were dried in a stream of air with the help of hair dryer in a wooden chamber with adequate ventilation. Densitometric scanning was performed using a Camag TLC scanner III in the reflectance- absorbance mode at 245 nm and operated by Wincats software (v 3.15, Camag). The source of radiation used was deuterium lamp emitting a continuous UV spectrum between 200 and 400 nm. Evaluation was performed by linear regression of peak areas determined by UV absorption as a function of sample analysis.

HPTLC METHOD AND CHROMATOGRAPHIC CONDITIONS

Preparation of standard stock solutions

Standard solutions were prepared by dilution of the stock solution with methanol to give solutions containing zolpidem in concentration range of 100-1000 ng/spot. Each

concentration was spotted six times on the TLC plate. The peak areas were plotted against the corresponding concentrations to obtain calibration graphs.

Prewashing of plates

Densitometric estimation was carried out on 20×10 cm pre-coated silica gel 60 F_{254} pre-coated plates from E. Merck. The plates were pre-washed with methanol, dried and activated for 30 min at 110°C.

Sample application

The standard and formulation samples of zolpidem were spotted on pre-coated TLC plates in the form of narrow bands of length 6 mm, with 10 mm from the bottom and left margin and 10 mm distance between two bands. Samples were applied under continuous drying stream of nitrogen gas at constant application rate of 150 ml/s.

Optimization of mobile phase

Initially various solvent systems like (a) water: methanol (5.0: 5.0; v/v) (b) chloroform: methanol (6.0: 4.0; v/v) (c) water: methanol (7.0: 3.0; v/v) (d) water: methanol (8.0: 2.0; v/v) in varying ratio were tried to separate and resolve spot of zolpidem from its impurities and other excipients of formulation. The mobile phase water: methanol: acetic acid (8.0: 2.7: 0.3; v/v/v) gave good resolution at 245 nm with R_f value 0.42±0.03 for zolpidem. Pre-saturation of TLC chamber with mobile phase for 30 min assured better reproducibility in migration of zolpidem and better resolution. Well defined spots were obtained when the chamber was saturated with the mobile phase for 30 min at room temperature (Fig. 2).

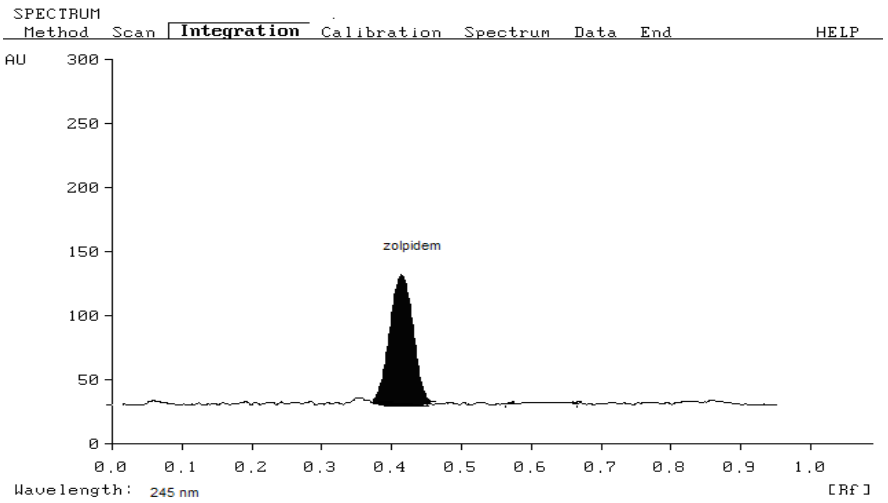

Fig.2: Densitogram of zolpidem.

METHOD VALIDATION

The developed HPTLC method was validated as per the ICH guidelines Q2 9(R1) for linearity, accuracy, precision, limit of detection, limit of quantification, repeatability, specificity and robustness.[8-11]

Linearity and calibration curve

Linearity was evaluated by determining six standard working solutions containing 100-1200 ng/ spot of zolpidem (n=6) twice in replicate for HPTLC. The calibration curves were developed by plotting peak area versus concentration with the help of Win-CATS software. The plate was developed in a twin through glass chamber, using 20 min chamber saturation time. The length of the run was 80 mm. The developed plates were air-dried. Scanning was performed in UV mode at 245 nm. The slit dimension was kept at 5 × 0.45 mm at scanning speed of 100 nm/s. After completion of scanning, peak areas were noted. Peak areas were plotted against corresponding concentration and least square regression analysis was performed to generate the calibration equation.

Precision

System repeatability was determined by six replicate applications and measurements of peak area for active compound and was expressed in terms of relative standard deviation (% RSD) and standard error (S.E). Method repeatability was obtained from R.S.D. value by repeating the assay of zolpidem as standard six times, on same day for (intra day precision). Intermediate precision was assessed by the assay; six sample sets on different days (inter day precision). The intra and inter day precision studies for determination of zolpidem was carried out at three different concentration levels 300, 600, 900 ng/spot.

Accuracy

Recovery studies of the drug were carried out for determining accuracy of the developed method. It was done by mixing known quantity of standard drug with the sample formulation and the contents were analyzed by the proposed method. Recovery studies were carried out at 80-120% levels. The percentage recovery and percentage RSD were calculated.

Limit of detection and limit of quantitation

In order to estimate the limit of detection (LOD) and lower limit of quantitation (LOQ), blank methanol was spotted six times following the same method. The signal to noise ratio (S/N) was determined. LOD was considered as 3:1 and LOQ as 10:1. Spotting for LOD was done

by taking different concentrations. The peak was detected at 10 ng/spot with a signal-to-noise ratio of 3:1. The LOQ was done by taking different concentrations .The peak was detected with quantifiable area with a signal-to-noise ratio of 10:1.

Specificity

The specificity of the method was ascertained by analyzing standard drug. The peak purity of zolpidem was assessed by comparing the spectra of the standard and the sample at three different levels, i.e. peak start (S), peak apex (S) and peak end (E) positions of the spot.

Recovery studies

Recovery studies were carried out by applying the method to preanalysed standard sample to which known amount of zolpidem corresponding to 80, 100, 120% of the label claim (Standard addition method). At each level of the amount, six determinations were performed and the results obtained were compared with the expected results.

Analysis of the marketed formulation

To determine the content of zolpidem in conventional tablets, ZOLDEM (label claim: 5 mg/tablet) the tablets were weighed and powder equivalent to 5 mg of zolpidem was weighed. The drug from the powder was extracted with methanol to ensure complete extraction of the drug, it was sonicated for 30 min and volume was made upto 100 ml. 4 µl of the resulting solution was diluted to 10 ml with methanol. The resulting solution was centrifuged at 3000 rpm for 5 min and supernatant was analyzed for drug content. 40 µl of the filtered solution 20 µg/ml was spotted and scanned into the HPTLC system the analysis was repeated in triplicate.

Robustness

As defined by ICH, the robustness of an analytical procedure describes to its capability to remain unaffected by small and deliberate variations in method parameters. The parameter selected for the robustness study were mobile phase composition, amount of mobile phase, temperature, relative humidity, plate pretreatment, time from spotting to chromatography, time from chromatography to scanning. It was observed that there were no marked changes in the densitograms which demonstrated that the developed HPTLC method is robust.

Stability in sample solution

Spot stability

The time the sample is left to stand in the solvent prior to chromatographic development can influence the stability of separated spots and are required to be investigated for validation. Two dimensional chromatography using same solvent system was used to find out any decomposition occurring during 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.[12-14]

RESULT AND DISCUSSION

Linearity

Linearity was evaluated by determining six standard working solutions containing 100-1200 ng/ spot of zolpidem (n=6) twice in replicate for HPTLC. Peak areas were plotted against corresponding concentration and least square regression analysis was performed to generate the calibration equation.

A representative calibration curve was obtained by plotting peak area of compound against the concentration over the range of 100-1000 ng/spot. The slope, intercept and correlation co-efficient values showed good correlation between regression coefficient and concentration of the drug. The results are depicted in Table 1.

Table 1: Linear regression data for the calibration curves.

Parameters	HPTLC
Linearity range	100-1000 ng/spot
r^2 ± S.D.	0.999 ± 0.21
Slope ± S.D.	0.18 ± 0.08
Intercept ± S.D[b]	0.12 ± 0.07

Precision

System repeatability was determined by six replicate applications and measurements of peak area for active compound and was expressed in terms of relative standard deviation (%RSD) and standard error (S.E). The intra and inter day precision studies for determination of zolpidem was carried out at three different concentration levels 300, 600, 900 ng/spot.

The repeatability of sample application and measurement of peak area were expressed in terms of % RSD and found to be 0.91 and 0.52 respectively. The % RSD for within and day to day analysis was found to be <2%. The results are depicted in Table 2.

Table 2: Results of precision studies.

Intra day			Inter day		
S.D	RSD	S.E	S.D	RSD	S.E
1.36	0.51	0.39	1.49	0.91	0.21

Accuracy

Recovery studies were carried out at 80-120% levels. The percentage recovery and percentage RSD were calculated.

The percent recovery of zolpidem was 99.12-100.10% (at 80%, 100% and 120% respectively), which was found to be satisfactory. The result of recovery studies indicated that the proposed method was accurate for estimation of drug in a tablet dosage form. The results are depicted in Table 3.

Table 3: Recovery studies of zolpidem tablet.

Excess drug added to the analyte (%)	Recovery (%)	(%) RSD	(%) SE
80	99.12	0.31	1.01
100	98.01	0.64	0.29
120	100.10	0.94	1.19

LOD and LOQ

In order to estimate the limit of detection (LOD) and lower limit of quantitation (LOQ), blank methanol was spotted six times following the same method. The signal to noise ratio (S/N) was determined. LOD was considered as 3:1 and LOQ as 10:1.

The signal to noise ratios 3:1 and 10:1 were considered as LOD and LOQ respectively. The LOD and LOQ were found to be 10 and 30 ng/spot.

Specificity

Specificity was checked for the interference of excipients in the analysis. When the Zoldem tablets were analysed, sharp and well defined peak was obtained at R_f 0.42±0.03 when scanned at 245 nm.

The peak purity of zolpidem was assessed by comparing the spectra of standard at peak start, peak apex and peak end positions of the spot i.e., r (start, middle) = 0.9997 and r (middle, end) = 0.9996. Good correlation (r=0.9997) was also obtained between standard and sample spectra of zolpidem. The densitogram of zoldem tablet is as shown in Fig.3.

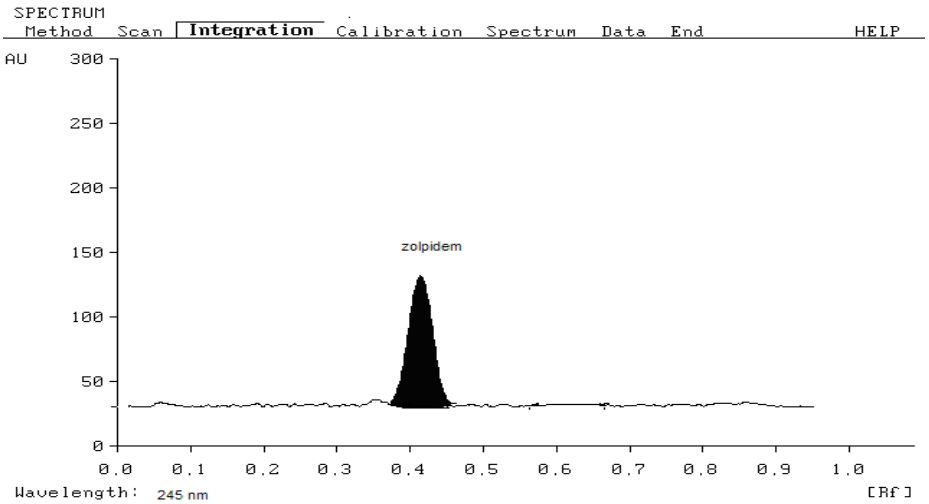

Fig.3: Densitogram of zolpidem formulation (Zoldem Tablet).

Robustness

Robustness of an analytical procedure describes to its capability to remain unaffected by small and deliberate variations in method parameters. The parameter selected for the robustness study were mobile phase composition, amount of mobile phase, temperature, relative humidity, plate pretreatment, time from spotting to chromatography, time from chromatography to scanning.

The standard deviation of peak area was calculated for each parameter and %R.S.D. was found to be less than 2%. The results are depicted in Table 4.

Table 4: Robustness testing for HPTLC method

PARAMETERS	SD OF PEAK AREA	% RSD
Mobile phase composition	2.48	2.10
Amount of mobile phase	1.24	1.61
Mobile phase composition	1.31	0.86
Temperature	1.34	1.08
Relative humidity	0.83	0.60
Plate pretreatment	1.17	1.40
Time from spotting to chromatography	1.72	0.99
Time from chromatography to scanning	1.66	0.31

(n = 6) Average of three concentrations 300, 600, 900 ng/spot.

Spot stability

The time the sample is left to stand in the solvent prior to chromatographic development can influence the stability of separated spots and are required to be investigated for validation. Two dimensional chromatography using same solvent system was used to find out any decomposition occurring during development.

Three different concentrations of zolpidem 300, 600, 900 ng/spot were prepared and stored at room temperature for 3 days, respectively. They were then applied on the same TLC plate, after development the densitogram were evaluated for additional spots if any. There was no instability in the sample solution. The results are depicted in Table 5.

Table 5: Stability of zolpidem in sample solutions.

PARAMETERS	HPTLC
S.D of Area	1.45
% R.S.D	1.32
S.E.	0.11

Method Validation

The developed HPTLC method is validated as per the ICH guideline. The summary of validation parameters is shown in Table 6.

Table 6: Summary of validation parameters.

PARAMETERS	HPTLC
Linearity Range	100-1000 ng/spot
Correlation Coefficient	0.999±0.21
Limit of detection	10 ng/spot
Limit of quantification	30 ng/spot
Recovery (n=6)	99.99±0.17
Precision (% RSD)	0.91 and 0.51%
Inter Day(n=6)	0.91
Intra Day(n=6)	0.51
Repeatability of application	1.52
Robustness	Robust
Specificity	Specific

CONCLUSION

The developed HPTLC method for quantitative analysis of zolpidem in pharmaceutical formulations is simple, precise, accurate, specific, reproducible and less economic without interference from the excipients. The method was validated in accordance with ICH guidelines. The method reduces analysis time compared with other methods and seems to be

suitable for routine analysis of pharmaceutical formulations in quality-control laboratories, where economy and speed are essential.

ACKNOWLEDGEMENT

Pharmaceutical grade zolpidem was kindly supplied as a gift sample by Shreeji Pharma International, Vadodara, India.

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