Research Article

Development and Validation of a Reversed-Phase HPLC Method for the Estimation of Zolpidem in Bulk Drug and Tablets

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In the present study an isocratic reversed-phase high-performance liquid chromatography method was developed for the estimation of zolpidem in bulk drug and pharmaceutical dosage forms. The quantification was carried out on C_{18} columns. A mixture of acetonitrile-ammonium acetate ($\text{pH} = 8.0$, 0.02 M) ($60:40 \text{ v/v}$) was used as the mobile phase, at flow rate of 1.0 mL/min and the determination wavelength at 245 nm . The retention time of zolpidem was found to be $3\text{--}5 \text{ min}$. The validation of the proposed method was carried out for specificity, linearity, accuracy, precision, limit of detection, limit of quantification, and robustness. The linear dynamic range was from 2.5 to $30 \mu\text{g mL}^{-1}$. Regression equation was found to be $y = 0.1416x + 0.0183$ with correlation coefficient $r = 0.9996$. The percentage recovery obtained for zolpidem was greater than 96.5% . Limit of quantification and limit of detection were found to be $2.5 \mu\text{g mL}^{-1}$ and $0.83 \mu\text{g mL}^{-1}$, respectively. The developed method can be used for routine quality control analysis of zolpidem in tablet formulations.

1. Introduction

Zolpidem, 2-(4-methyl phenyl) N, N-6-trimethyl-imidazol [1, 2- α] pyridine-3-acetamide, an imidazopyridine derivative, is a nonbenzodiazepine hypnotic indicated for the short-term treatment of insomnia. Zolpidem (Figure 1) behaves as a sleep inducer without the muscle relaxant and anticonvulsant effects of the benzodiazepines. It improves sleep patients with insomnia.

The free base has a molecular weight of 307.4 , with the salt form (zolpidem hemitartrate) having a formula weight of 764.9 . The salt has an appearance of a white to off-white powder, with a melting point of $193\text{--}197^\circ\text{C}$. The tartrate salt is slightly soluble in water (23 g/L at 20°C), sparingly soluble in methanol, and practically insoluble in methylene chloride. The Ultraviolet (UV) spectrum of zolpidem in 0.1 N HCL exhibits the λ maximum of 294 nm [1–6].

Zolpidem is not a difficult drug to extract, detect over the therapeutic range, or detect at overdose levels. It has been measured in various biological specimens using a variety of different analytical techniques. Several analytical methods have been reported for quantification of zolpidem

in human body fluids and organ samples including capillary electrophoresis (CE) [7], GC [8–10], HPLC [1, 3, 11–17], GC/MS [9, 18, 19], LC/MS [20], LC/MS/MS [21–23], and radioimmunoassay [24]. In the HPLC methods, the sensitivity of zolpidem is higher with fluorimetric detection than with UV detection [25]. Among these technologies, LC/MS and GC/MS are very expensive and are not often affordable for a common laboratory nowadays, and the derivative procedure made GC difficult to be a robust tool for monitoring zolpidem in many samples. Hence, it is a great selection to develop sufficiently selective and sensitive analytical method in terms of often used instruments such as HPLC-UV.

The main objective of the present work was to develop simple, fast, inexpensive, sensitive, and accurate method which could be applied to analyses of zolpidem in pure form and in pharmaceutical dosage form.

2. Experimental

2.1. Equipment. The HPLC apparatus was a Waters chromatographic system 1515 isocratic HPLC pump equipped with an injection valve $20 \mu\text{L}$ and Waters 2487 UV-Visible

FIGURE 1: Structure of zolpidem.

detector. A reversed phase C_{18} column (Waters Xterra, 4.6×250 mm, particle size $5 \mu\text{m}$) was used. The column oven temperature was set at $30 \pm 2^\circ\text{C}$. Peak area integration was performed using Breeze software version 3.30.

Samples were injected using $25 \mu\text{L}$ Hamilton analytical syringe.

2.2. Chemicals and Reagents. HPLC grade acetonitrile, ammonium acetate (proanalysis), and orthophosphoric acid and ammonium solution 25% (pro analysis) were purchased from Merck (Germany). Triple distilled water from the Milipore equipment was used to prepare all solutions. Authentic samples of zolpidem were obtained from Hexal pharmaceuticals (Germany).

The buffer solution was prepared by dissolving 1.54 g ammonium acetate in 1000 mL of triple distilled water and the pH was adjusted to 8 by addition of ammonium solution 25%.

2.3. Preparation of Standard Solutions and Samples. An accurate weighted zolpidem reference standard was transferred to a 50 mL volumetric flask and dissolved in mobile phase to make solution of $20 \mu\text{g mL}^{-1}$. This solution contains about 0.02 mg of zolpidem RS per mL.

Twenty tablets were finely powdered and weighed; the average tablet weight was determined and weighed into 25 mL volumetric flask and dissolved in mobile phase. To dissolve it, the solution was sonicated into an ultrasonic bath for 5 min, and transfer 2 mL of this solution to a 20 mL volumetric flask, dilute with mobile phase to volume, and mix [12]. Then this solution that contains 0.02 mg/mL of zolpidem was filtered through a $0.45 \mu\text{m}$ PTFE syringe filter (Sartorius) and was used as sample solution.

For process control, $25 \mu\text{L}$ of sample were pipetted accurately into 50 mL volumetric flasks and subsequently diluted to the desired volume with mobile phase. The solutions were sonicated into an ultrasonic bath for 1 min and filtered through a $0.45 \mu\text{m}$ PTFE syringe filter (Sartorius) and then $20 \mu\text{L}$ were injected into the HPLC apparatus.

2.4. Calibration Procedure. The calibration curve was obtained with six concentrations of standard solution 2.5

FIGURE 2: Chromatograms of a solution containing zolpidem at a concentration of $20 \mu\text{g mL}^{-1}$ at the described chromatographic conditions.

to $30 \mu\text{g mL}^{-1}$; solutions were prepared in triplicate. The linearity was evaluated by linear regression analysis, which was calculated by the least square regression method. Before injection of solution, the column was equilibrated for 60 min with mobile phase flowing through the system. Three determinations were carried out for each solution. Peak area was recorded for all the solutions. The correlation graph was constructed by plotting the peak area obtained at the optimum wavelength of detection versus the injected amounts.

2.5. Chromatographic Conditions. The mobile phase was a mixture of acetonitrile/ammonium acetate buffer 0.02 M, pH = 8, (60 : 40 v/v). The flow rate was 1 mL min^{-1} . The UV detector wavelength was set at 245 nm and the column oven temperature was set at $30 \pm 2^\circ\text{C}$.

3. Results and Discussion

3.1. System Suitability. The chromatographic separation, as explained above, was carried out with HPLC to evaluate the chromatographic parameters including retention factor (k), asymmetry of the peak, and tailing factor. Figure 2 showed that no interference peaks were observed in the representative chromatogram. The retention factor (k) of the zolpidem peak, the tailing factor parameter, and USP Plate Count were 4.4, 1.1 and >7500 , respectively. It was concluded that the developed method is the optimum according to the studied parameters. The resulted retention factor was within the range of accepted value (>2). The tailing factor to be controlled was within the limits established by these guidelines.

It can be seen in Figure 2, which shows the chromatogram obtained according to these optimized conditions, good peak asymmetry in a reasonable run time, that is, 4 min. Alternatively, different gradient elution programs using acetonitrile as organic modifier were tested and used

TABLE 1: Accuracy for zolpidem.

Nominal concentration ($\mu\text{g mL}^{-1}$)	Mean measured concentration ($\mu\text{g mL}^{-1}$) ^a	Recovery (%) ^a	RSD (%) ^b
10	9.67 (± 0.3)	96.45	0.07
20	19.6 (± 0.4)	98.00	1.00
30	31.3 (± 0.2)	104.3	0.30
Mean		99.60	0.46

^a Mean of six determinations.^b RSD: relative standard deviation.

photodiode array detector was published [6]. The HPLC analyses were performed using an isocratic mobile phase consisting methanol-acetonitrile and 26 mM sodium acetate buffer (pH 2.0) containing 0.26 mM tetrabutyl ammonium phosphate (13 : 10 : 77 v/v/v) and flow rate was set at 0.3 mL min^{-1} in another published paper [12].

Therefore, the main objective of the present work can be applied properly to its intended purpose, and compared with past published papers HPLC methods are very easy, simple, fast, and inexpensive.

3.2. Stability of the Solution. Results obtained in the study of stocks solutions of zolpidem in mobile phase were found to be stable for 5 days (120 h), when kept in refrigerator.

4. Method Validation

4.1. Specificity. Specificity described as the ability of a method to discriminate the analyte from all potential interfering substances was evaluated by preparing the analytical placebo and it was confirmed that the signal measured was caused only by the analytes. A solution of an analytical placebo (containing all the ingredients of the formulation except the analyte) was prepared according to the sample preparation procedure and injected to HPLC. A mixture of the inactive ingredients (placebo) before (Figure 3(a)) and after being spiked with standards (Figure 3(b)), standard solutions (Figure 3(c)), and the commercial pharmaceutical preparations including zolpidem (Figure 3(d)) were analyzed by the proposed method, to identify the interference by these recipients. The obtained results show that the peak of analytes was pure and recipients in the formulation did not interfere with the analyte.

4.2. Accuracy and Recovery Studies. Accuracy was studied using three different solutions, containing 10, 20, and $30 \mu\text{g mL}^{-1}$ of zolpidem. Recovery data were reported in Table 1. The obtained values were within the range of 96.45 and 104.3%.

4.3. Precision. The precision of an analytical procedure expresses the closeness of agreement between a series of measurement obtained from multiple sampling of the same homogenous sample under the prescribed conditions. The system precision is a measure of the method variability that

can be expected for a given analyst performing the analysis and was determined by performing six replicate analyses of the same working solution. The relative standard deviation (RSD) obtained for zolpidem was 0.46 (Table 1).

The intra- and interday variability or precision of data is summarized in Table 2. The intraday precision of the developed LC method was determined by preparing the tablet samples of the same batch in ten determinations with three concentrations, expressed as a percentage of the label claim, which were used to evaluate the method precision. The intraday precision was also determined by assaying the tablets in triplicate per day for consecutive 3 days. The results indicated the good precision of the developed method (Table 2).

4.4. Linearity. The linearity of the proposed method was estimated by regression analysis at six concentration levels in the range of $2.5\text{--}30 \mu\text{g mL}^{-1}$. The slope and intercept of calibration curves are shown in Table 4. The calibration curve was obtained by applying linear regression model based on the least square method.

4.5. Limit of Detection (LOD) and Limit of Quantification (LOQ). LOD and LOQ of zolpidem were determined by calibration curve method [26]. Solutions of zolpidem were prepared in the range of $2.5\text{--}30 \mu\text{g mL}^{-1}$ and injected in triplicate. Average peak area of three analyses were plotted against concentration. The LOD and LOQ were calculated using the following equations:

$$\begin{aligned}\text{LOD} &= \frac{3.3 \times S_a}{b}, \\ \text{LOQ} &= \frac{10.0 \times S_a}{b},\end{aligned}\quad (1)$$

where S_a is residual variance due to regression; b is slope. The obtained LOD and LOQ for zolpidem were 0.83 and $2.5 \mu\text{g mL}^{-1}$, respectively (Table 3).

4.6. Robustness. Robustness relates to the capacity of the method to remain unaffected by small but deliberate variations introduced into the method parameters. Several experimental parameters as changes of pH of the mobile phase, flow rate, percentage of acetonitrile in the mobile phase, column oven temperature, and detection wavelength were varied around the value set in the method to reflect changes in different test environments. Analysis was carried out in triplicate and only one parameter was changed in the experiments at a time. The degree of reproducibility of the results obtained as a result of small deliberate variations in the method parameters has proven that the method is robust (Table 4).

4.7. Analysis of Pharmaceutical Preparations. Developed and validated method was applied to determination of zolpidem in ten different pharmaceutical preparations. These preparations have the same amount of zolpidem (5 mg). Each pharmaceutical preparation was analyzed by performing eight independent determinations. Satisfactory results were

TABLE 2: Intra- and interday assay precision data ($n = 10$).

Standard concentration ($\mu\text{g mL}^{-1}$)	Measured Concentration					
	mg/tab	Intraday Recovery (%)	RSD (%)	mg/tab	Interday Recovery (%)	RSD (%)
10.8	4.93	98.7	0.2	4.95	99.05	1.6
21.6	5.13	102.7	0.3	5.12	102.50	1.5
32.4	5.18	103.6	0.3	5.09	101.80	0.8

Nominal concentration of sample solution was $20 \mu\text{g mL}^{-1}$ and tablets were 5 mg.

FIGURE 3: The chromatograms of (a) tablet excipients (placebo), (b) tablet excipients after being spiked with standard, (c) standard solutions, and (d) commercial pharmaceutical preparation at the described chromatographic conditions.

TABLE 3: Results of the data analysis for the quantitative determination of zolpidem by the proposed method.

Statistical parameter	HPLC
Concentration range ($\mu\text{g mL}^{-1}$)	2.5–30
Regression equation	$y = 0.1416x + 0.0183$
Correlation coefficient (r)	0.9996
Standard error on estimation (S_e)	0.049
Standard deviation on slope (S_b)	0.00199
Standard deviation on intercept (S_a)	0.0356
Limit of detection (LOD) ($\mu\text{g mL}^{-1}$)	0.83
Limit of quantification (LOQ) ($\mu\text{g mL}^{-1}$)	2.5

TABLE 4: Result of robustness study.

Factor	Level	Mean	% RSD
pH of mobile phase	6.3	5.03	0.4
	8.0	5.01	0.8
	9.67	5.06	0.3
Flow rate (mL/min)	0.5	5.10	0.6
	1.0	5.15	0.8
	1.5	5.12	0.2
	2.0	5.16	0.4
Column oven temperature ($^{\circ}\text{C}$)	25	5.03	0.8
	35	5.07	0.1
	45	5.10	0.4
% acetonitrile	(55/45)	4.98	0.5
	(60/40)	5.01	0.3
Detection wavelength (nm)	241	5.01	0.4
	243	5.04	0.5
	245	5.11	0.2
	250	5.00	0.2

obtained for each compound and were found to be in agreement with label claims (Table 5).

The proposed method was compared to the UV spectrophotometric method, to verify the results obtained from HPLC. A calibration equation was obtained in the concentration range 2–10 $\mu\text{g mL}^{-1}$ at a wavelength of 245 nm by using methanol as blank. The relation between absorbance (A) and concentration of zolpidem (C) was [$y = 0.1231x - 0.0004$, $r = 0.9996$]. The tablet dosage from analysis results was found to be 5.37 ± 0.07 (mean $\pm$ SD; $n = 6$) by UV spectrophotometric method. High reproducibility and insignificant difference between the two methods were obtained at the 0.05 probability level according to t - and F -tests.

5. Conclusions

A simple, specific, linear, precise, and accurate RP-HPLC method has been developed and validated for quantitative determination of zolpidem in tablet formation. The method is very simple and specific for zolpidem (chromatograms show no other peaks) and excipient peaks with total run time of 5 min, which make it especially suitable for routine quality control analysis work.

TABLE 5: Data from the analysis of commercial tablets by HPLC and UV method.

Company	HPLC method		UV method	
	mg/tab	Recovery %	mg/tab	Recovery %
Hexal ^a	10.08	100.7	10.10	100.9
Sandoz ^a	10.60	105.5	10.16	101.7
Aboreyhan ^b	5.06	101.3	5.30	105.6
Amin ^b	5.23	104.6	5.20	104.0
Bakhtar bio shimmy ^b	4.93	98.7	5.30	105.6
Jalinous ^b	4.94	98.8	5.07	101.5
kish medi pharma ^b	5.10	102.2	5.12	102.6
kimidaro ^b	4.99	99.9	5.50	109.9
loghman ^b	5.50	109.7	5.47	109.5
Osvah ^b	5.20	103.3	4.94	98.8

Nominal concentration of each company was 20 $\mu\text{g mL}^{-1}$.

^aNominal mg per tab of these companies (Germany) was 10 mg.

^bNominal mg per tab of these companies was 5 mg.

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