Validated Stability Indicating HPLC Method for Determination of Zolpidem in the Presence of Its Degradation Products

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Abstract: Zolpidem is a hypnotic agent used for the treatment of insomnia. In this study a stability indicating HPLC method was developed for the determination of zolpidem in the presence of its degradation products. Stress degradation of zolpidem was performed under acidic, alkaline, oxidative, heat and photolytic conditions. Separation of zolpidem and its degradation products were performed on a Nova-Pak CN column using KH_2PO_4 30 mM and acetonitrile (65: 35, v/v at pH 6) as mobile phase. Acceptable linearity (r^2 >0.999) and precision (CV value <1.5%) were achieved over the concentration range of 1-20 μg/mL.

Degradation of zolpidem was observed under acidic, alkaline, oxidative conditions and also exposure to UV light. The proposed method was used for assay determination of zolpidem tablets with no interfering from excipients.

Keywords: Zolpidem, HPLC, Stability indicating, Degradation.

INTRODUCTION

Zolpidem, 2- (4-methylphenyl)-N, N, 6- trimethyl-imidiazo [1, 2-a] pyridine-3- acetamide (Fig. **1**), is a non-benzodiazepine hypnotic agent related to imidazopyridine class. Zolpidem is currently formulated as a hemitartrate salt in tablets. Zolpidem is an agonist for benzodiazepine site of the $GABA_A$ receptor and binds preferentially to the omega-1 benzodiazepine receptor subtype which seems to mediate hypnotic effect. Zolpidem is used for the short-term treatment of insomnia without the muscle relaxant or anticonvulsant effects of the benzodiazepines [1, 2].

Several HPLC [3-7] and LC/MS [8,9] methods have been published for determination of zolpidem in biological fluids. Also HPLC [10, 11], spectrophotomertic [12] and potentiometric [13] methods have been reported for determination of zolpidem in bulk powder or pharmaceutical dosage forms.

According to the International Conference on Harmonization (ICH) guidelines and USP, information on stability of an active substrate under hydrolytic, oxidative, heat and photolytic conditions is necessary to develop an assay method for the determination of a pharmaceutical dosage form. Based on our knowledge from literature survey, no comprehensive study has been published for determination of zolpidem in the presence of its degradation products in different conditions.

The objective of this study was to develop and validate a stability indicating HPLC method according to the ICH guidelines to be used for determination of zolpidem in the presence of its degradation products.

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MATERIALS AND METHODS

Materials

Zolpidem tartrate was from Centour, India (batch No: 20062502). Other pro-analysis chemicals and HPLC grade solvents were from Merck (Darmstadt, Germany). HPLC grade water was obtained by a Milli-Q purification system (Millipore, Milford, MA, USA).

Instrumentation

A Waters HPLC system consisted of an isocratic pump (Model 515), an autosampler (Model 710 plus) and a variable UV-Vis detector (Model 480) was used. The HPLC data were processed using a multi-channel Chrom & Spec software for chromatography, version 1.5 x. The light sources were a l00 W Tungsten lamp (visible light) and a low-pressure Mercury lamp 200 W (UV light) with λ max at around 254 nm. A dry air oven (Melag, Germany) was used for some stress reactions.

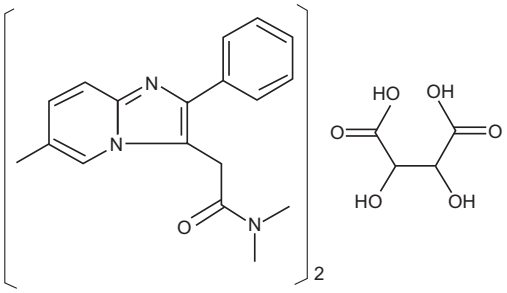

Fig (1). Chemical structure of zolpidem.

Chromatographic Conditions

Chromatographic separation was carried out by using a Nova- Pak CN 4 μm column (150 mm × 3.9 mm, Waters) and a mixture of KH_2PO_4 30 mM and acetonitrile (65:35, v/v), pH adjusted to 6, as mobile phase at a flow rate of 1 mL/min. The mobile phase was prepared daily and filtered through a 0.45 μm Teflon membrane filter (Millipore, Milford, MA, USA) and degassed under sonication for 10 min. The UV-Vis detector was set at 240 nm and all determinations were performed at ambient room temperature.

Degradation studies

The degradation studies were performed using an initial concentration of 500 μg/mL under the following conditions.

- For acidic degradation, a solution of zolpidem tartrate in 5 M HCl was heated on a water bath at 95 °C for 3 h.

- The alkaline degradation was performed on a solution of zolpidem tartrate in 1 M NaOH at 95 °C for 1 h.

- For oxidative degradation studies, a solution of zolpidem tartrate in 3% H_2O_2 was kept at 70°C for 3 h.

- For light stress studies, about 100 mg of zolpidem tartrate was spread on a watch glass in a layer less than 2 mm in thickness and exposed to visible light and UV light. A solution of zolpidem tartrate in water (500 μg/mL) in Pyrex flasks was also exposed to light. The samples were exposed to irradiation at 15 cm from the light source in a 40 cm × 30 cm × 30 cm chamber for 5 days.

- For thermal stress studies, zolpidem tartrate powder was exposed to dry heat of 80 °C in an oven for 6 days. A solution of zolpidem tartrate (500 μg/mL of water) was also kept in a dry oven at 80 °C for 5 days.

The solutions of acidic or alkaline conditions were neutralized by adding appropriate amounts of sodium hydroxide or hydrochloric acid and injected to the HPLC system after dilution with mobile phase to reach the stated concentration of 25 μg/mL.

An accurately weighed portion of the powder samples was also dissolved in the mobile phase and diluted with mobile phase to reach the concentration of 25 μg/mL before injection to the HPLC system. The peak area of the zolpidem tartrate in all degradation conditions was compared with freshly prepared samples at the starting concentration and the percentage of degradation was calculated. All experiments were performed in triplicate and mean data calculated.

Method validation

The linearity test solutions were prepared in six series at the concentrations of 1, 2, 4, 10, 15 and 20 μg/mL of mobile phase. The solutions were injected to the HPLC system and the calibration curves were constructed by plotting the peak area against concentration.

To evaluate the within-day and between-day precision and accuracy, standard solutions of zolpidem tratrate in mobile phase at 1, 4 and 20 μg/mL were injected to the HPLC system in triplicate in one day and three consecutive days. The concentration of each solution was measured using a calibration curve in the range of 1-20 μg/mL.

For robustness studies, the influence of the percent composition of organic solvent and the pH of the mobile phase were evaluated on the retention time and peak area of a standard solution of zolpidem tartrate.

Application of the Method

Twenty zoplidem tartrate tablets were powdered finely. An accurately weighed portion of powder equivalent to one tablet was transferred to a 100 mL volumetric flask and 50 mL of distilled water was added. After 15 min sonication, the flask was adjusted to the volume, and the solution was injected to the HPLC system after filtration through a 0. 45 μm polypropylene syringe filter (Teknokroma, Spain) and ten times dilution. The peak area of this solution was compared with a standard solution of zolpidem tartrate at the same concentration.

The drug release profile of zoplidem tablets was also measured by using a dissolution apparatus (Erweka, Heusenstamm, Germany) at 37 ± 0.5 °C. The dissolution medium was 900 mL of 0.01 M HCl (pH 2) and paddle apparatus at rotation speed of 50 rpm was used. Samples of 2 mL were drawn at 5, 10, 20 and 30 min and injected to the HPLC system after filtration. The percent drug released was measured by comparison of the resulted peak area with the peak area of a standard solution of zolpidem tartrate.

Relative Recovery

Standard addition method was used to evaluate the recovery of zolpidem tartrate from dosage forms. Stock standard solution of zolpidem tartrate was added to a 100 mL volumetric flask containing an amount of powdered tablet equivalent to 50% of a tablet. The same procedure for assay method of zoplidem tablets was performed. The peak area of the resulted solution was compared with a standard solution of zoplidem tartrate at the same concentration level and the relative recovery was calculated.

RESULTS AND DISCUSSION

Chromatographic Conditions

Using Nova-Pak C_{18}, Symmetry C_{18}, Nova-Pak C_8 or Nova- Pak CN and varying mobile phase systems, the best results were observed by a Nova-Pak CN column and a mixture of KH_2PO_4 30 mM and acetonitrile (65:35) at pH 6. Well-resolved peaks, acceptable symmetry and suitable retention times were achieved. A typical chromatogram is observed in Fig. (**2a**). The system suitability parameters calculated for six replicate injections were also within the acceptable range (Table **1**).

Validation of the developed method

The statistical data for six series of calibration curves of zolpidem tartrate in the range of 1-20 μg/mL are listed in Table **2**. The limit of quantification (LOQ) of the developed method with CV<1.5% was found to be 1 μg/mL.

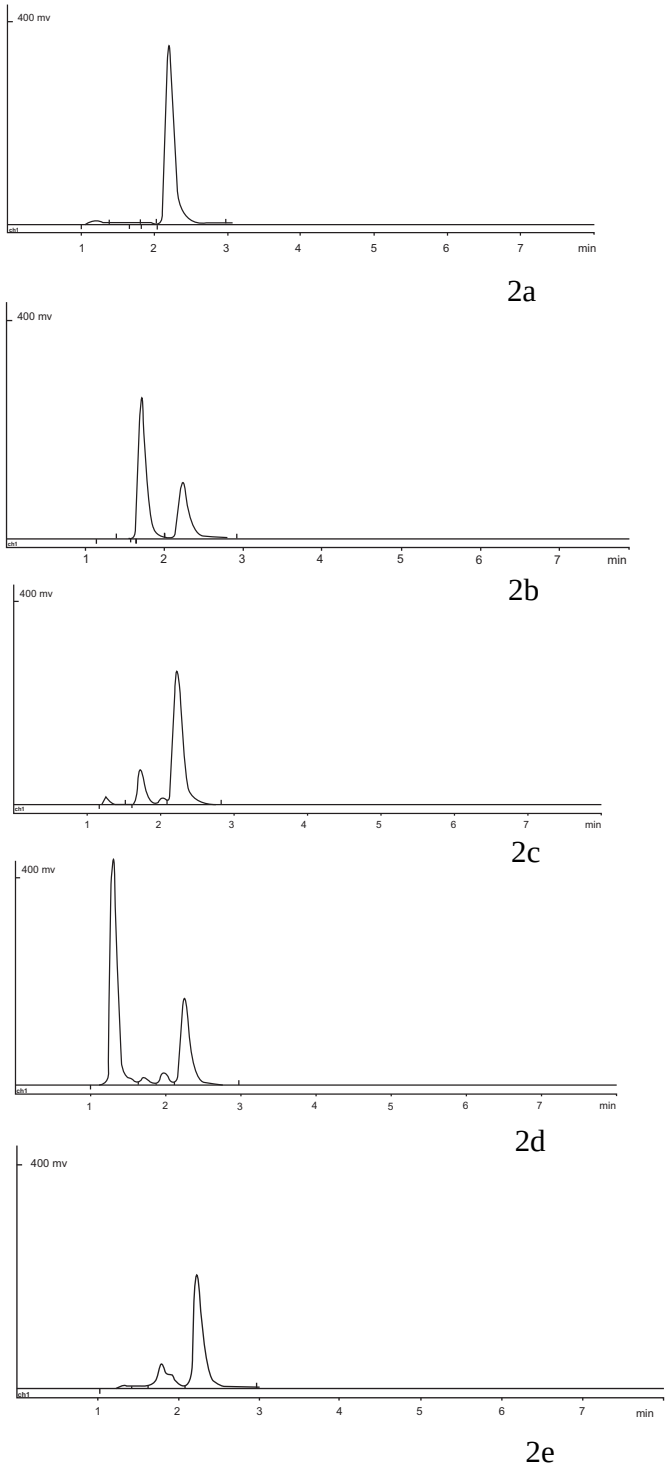

Fig (2). Typical chromatograms obtained from stability studies of zolpidem tartrate . (**a)** Zolpidem tartrate standard solution (25 µg/mL); (**b**) Zolpidem tartrate solution in 1 M NaOH after 1h at 95°C; **(c)** Zolpidem tartrate solution in 5 M HCl after 1 h at 95°C; **(d)** Zolpidem tartrate solution in 3% H_2O_2 after 1 h at 70°C; (**e)** Zolpidem tartrate solution in water after 2 days exposure to UV light.

The within-day and between-day precision and accuracy results at three different concentrations are shown in Table **3**.

Table 1. System Suitability Parameters

Parameters	Found	Acceptable Limits
USP theoretical plates (n = 6)	3600	N>1500
USP tailing factor (n = 6)	1.08	T<1.5
Repeatability (t_R) (n = 6)	0.25	RSD<1%
Repeatability (peak area)(n = 6)	0.63	RSD<1%

t_R: Retention time (min); N: Theoretical plate; T: Tailing factor; RSD: Relative Standard Deviation

Table 2. Statistical Data of Calibration Curves of Zolpidem Tartrate (n = 6)

Parameters	Results
Linearity range	1-20 µg/mL
Regression equation	y = 113.06 x-2.88
Standard deviation of slope	0.55
Relative standard deviation of slope (%)	0.48
Standard deviation of intercept	0.41
Correlation coefficient (r^2)	0.9995

Also comparison of the assay results for Zolpidem tartrate tablets by two analysts using two different HPLC systems showed CV values <2%.

Sufficient robustness of the method was indicated by the results of the experiments after small condition changes. Peak area values were not influenced more than 2% in all different conditions. The changes in retention times using different mobile phase composition has no effect on separation and quantification.

The relative recovery of zolpidem tartrate after standard addition method to tablet powder was about 100% with no interference from tablet excipients.

Methanolic stock standard solution of zolpidem tartrate was stable for at least 7 days after storage at 4°C. Also the drug solution in mobile phase was relatively stable for 24 h at room temperature with a recovery of 99%.

Application of the method

The proposed method was used for determination of zolpidem tartrate in Stilnox®10 mg (Sanofi aventis, France). The results were acceptable (10.16 ± 0.11 mg per tablet) and in good agreement with the label claims.

The proposed HPLC method was also used for determination of zolpidem tartrate in dissolution medium. The dissolution profile of the drug (n=6) showed 95% release within the first 10 min (Fig. **3**).

Degradation studies

The obtained results of degradation of zolpidem tartrate under different conditions are summarized in Table **4**. Also typical chromatograms of degradation of zolpidem tartrate under various conditions are shown in Figs. (**2b**-**e**).

Table 3. Precision and Accuracy of the Method for Determination of Zolpidem Tartrate (Three Sets for 3 Days)

Concentration Added (μg/mL)	Concentration Found (μg/mL)	CV (%)	Error (%)
Within-day (n = 3)			
1.00	1.00 ± 0.01	1.48	1.00
4.00	4.01 ± 0.03	0.69	0.25
20.00	20.21 ± 0.03	0.15	1.05
Between -day (n = 9)			
1.00	1.01 ± 0.01	1. 17	1.00
4.00	3.99 ± 0.04	1.06	-0.25
20.00	20.13 ± 0.20	1.00	0.65

Table 4. The Results of the Stress Degradation Tests Using Different Conditions

Stress Test Condition	Solvent	Temperature	Time	% of Zolpidem
Acidic	1 M HCl	95 °C	4 h	90.2
	2 M HCl	70 °C	3 h	97.0
	2 M HCl	95 °C	3 h	75.7
	5 MHCl	95 °C	3 h	62.9
Alkaline	1 M NaOH	95 °C	1 h	35.1
Oxidative	3% H_2O_2	70 °C	1 h	54.6
	10% H_2O_2	70 °C	1 h	37.6
Photolytic				
UV light	Solid form	Room temperature	5 days	90.2
UV light	Water	Room temperature	5 days	45.0
Visible light	Solid form	Room temperature	5 days	84.6
Visible light	Water	Room temperature	5 days	83.0
Heat	Solid form	80 °C	5 days	85.0
	Water	80 °C	5 days	85.7

The degradation of zolpidem tartrate in acidic conditions has been reported before [10], but the characterization of the degradation product and also degradation under other conditions was not studied.

The degradation of zolpidem tartrate in acidic conditions was studied using different strength of hydrochloric acid. As shown in Table **4** the degradation was accelerated by increasing the concentration of hydrochloric acid or reaction temperature.

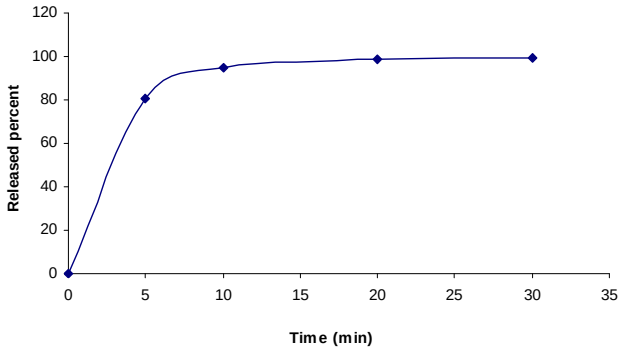

Fig (3). Dissolution profile of 10 mg Stilnox tablets (n=6), using 0.01 M HCl (pH=2) as dissolution medium and paddle at 50 rpm.

The concentration of zolpidem tartrate decreased with time on heating at 95°C in 1 M NaOH or 5M HCl, forming a new peak at retention time of 1.7, the concentration of which increased with time. The degradation product in acidic and alkaline medium was similar but the rate of hydrolysis in acid conditions was slower than alkaline conditions.

The degradation of zolpidem tartrate in 3% and 10% hydrogen peroxide at 70°C after 1 h was about 55 % and 38% respectively. Two unknown peaks were detected at retention times of 1.7 and 2 min.

The drug powder was relatively stable after exposure to visible or UV light and also under dry heat for 5 days. In these conditions about 15% decrease in peak area was observed.

Significant degradation of zolpidem tartrate solution (55% degradation) was observed upon exposure to UV light, leading to the formation of several unknown peaks. The ma-

jor unknown degradation product was detected at retention time of 1.8 min.

CONCLUSION

The developed method was simple, accurate and reproducible for determination of zolpidem tartrate in the presence of its degradation products in acidic, alkaline, oxidative and photolytic conditions. The proposed method could also be used for routine quality control studies, such as dissolution and assay of zolpidem dosage forms.

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CONFLICT OF INTEREST

None declared.

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