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RESEARCH ARTICLE

Development and Validated of Stability-Indicating RP-HPLC Method for the Analysis of Zolpidem Tartrate in Tablets

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Abstract: A validated stability-indicating high-performance liquid chromatographic technique was developed for the determination of Zolpidem tartrate in pharmaceutical dosage forms. Chromatographic separation was performed on Shimadzu Model CBM-20A/20 Alite, using water: methanol: acetic acid(25: 75: 0.1, v/v) as mobile phase with a flow rate of 1.2 mL/min. Zolpidem tartrate was subjected to stress conditions (acidic, alkaline, oxidation, thermaland photolytic) and the method was validated as per ICH guidelines.

Keywords: Zolpidem tartrate, RP-HPLC, Stability-indicating, ICH

Introduction

Zolpidem tartrate (ZPT), chemically known as N, N, 6-Trimethyl-2-ptolyl-imidazo (1,2-a) pyridine-3-acetamide L-(+)-tartrate (2:1) (Figure 1) is an imidazo pyridine derivative, is a non-benzodiazepine hypnotic agent binds preferentially to one benzodiazepine receptor subtype ω-1 bezodiazepine-1thought to mediate hypnotic effects[1]. This combines a rapid onset with a short duration of action. Zolpidem tartrate behaves as a sleep inducer without the muscle relaxant and anticonvulsant effects of the benzodiazepines. The hypnotic actions of Zolpidem, like benzodiazepine hypnotics, are mediated at the benzodiazepine recognition site of the GABAA receptor complex[2-4]. However, the neuro pharmacological profile of Zolpidem tartrate is somewhat different from that of most benzodiazepines[5-6]. Zolpidem binds with low affinity to a α5 –containing GABAA–receptor subtypes[7].

Literature survey revealed that Zolpidem tartrate was determined by liquid chromatographic methods[8-19], LC-MS[20-21], GC[22-23], GC-MS[24], capillary electrophoresis[25], UV-Visible spectroscopy[26-32] and HPTLC-LC[33].

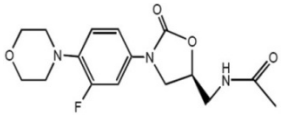


Figure 1. Chemical structure of Zolpidem tartrate (ZPT)

Experimental

Analytical grade reagents were used. Zolpidem tartrate was supplied as gift sample from Dr. Reddy's Labs (India) India. Zolpidem tartrate (ZPT) stock was prepared by dissolving 25 mg of the drug in 25 mL of methanol in a volumetric flask (1000 µg/mL) and working standard solutions were obtained by proper dilution of this stock solution with mobile phase.

Zolpidem tartrate (ZPT) is available commercially as tablets with brand names ZOLINOX® (Ranbaxy Laboratories, India) (Label claim: 7.5 mg) and AMBIEN® (Dr. Reddy's Laboratories, India) (Label claim: 10 mg) and twenty tablets from each brand were procured from the local market.

Instrumentation

Chromatographic separation was achieved by using C18 column (250 mm × 4.6 mm i.d., 5 µm particle size) for HPLC system of Shimadzu Model CBM-20A/20 Alite, equipped with SPD M20A prominence photodiode array detector, maintained at 25 ºC.

Chromatographic conditions

Isocratic elution was performed using water: methanol: acetic acid (25:75: 0.1, %v/v) as mobile phase. The overall run time was 10 min. with flow rate 1.2 mL/min with UV detection at 254 nm. 20 µL of sample was injected into the HPLC system.

Preparation of stock and sample solution

Zolpidem tartrate stock solution (1000 µg/mL) was prepared by weighing accurately 25 mg of Zolpidem tartrate in a 25 mL volumetric flask with mobile phase. Working standard solutions were prepared on daily basis from the stock solution with mobile phase and filtered through 0.45 µm membrane filter prior to injection.

Method validation

The method was validated for system suitability, linearity, limit of quantitation (LOQ), limit of detection (LOD), precision, accuracy, selectivity and robustness[34].

Linearity

Linearity test solutions for the assay method were prepared from a stock solution at different concentration levels (0.5-200 µg/mL) of the assay analyte concentration and 20 µL of each solution was injected in to the HPLC system and the peak area of the chromatogram obtained was noted. The calibration curve was plotted by taking the concentration on the x-axis and the corresponding peak area on the y-axis. The data was treated with linear regression analysis method.

The limit of quantification and limit of detection were based on the standard deviation of the response and the slope of the constructed calibration curve, as described in ICH guidelines Q2 (R1)[34].

Precision study

The intra-day precision of the assay method was evaluated by carrying out 3 independent assays of a test sample of Zolpidem tartrate at three concentration levels (20, 50 and 100 µg/mL) against a qualified reference standard. The %RSD of three obtained assay values at three different concentration levels was calculated. The inter-day precision study was performed on three different days *i.e*. day 1, day 2 and day 3 at three different concentration levels (20, 50 and 100 µg/mL) and each value is the average of three determinations. The % RSD of three obtained assay values on three different days was calculated.

Accuracy study

The accuracy of the assay method was evaluated in triplicate at three concentration levels (80, 100 and 120%) and the percentage recoveries were calculated. Standard addition and recovery experiments were conducted to determine the accuracy of the method for the quantification of Zolpidem tartrate in the drug product. The study was carried out in triplicate at 18, 20 and 22 µg/mL. The percentage recovery in each case was calculated.

Robustness

The robustness of the assay method was established by introducing small changes in the HPLC conditions which included wavelength (252 and 256 nm), percentage of methanol in the mobile phase (73 and 77%) and flow rate (1.1 and 1.3 mL/min). Robustness of the method was studied using six replicates at a concentration level of 100 µg/mL of Zolpidem tartrate.

Analysis of marketed formulations

The content of 20 tablets of each brand was mixed and quantity equivalent to 25 mg of drug weighed accurately and dissolved in mobile phase in a 25 mL volumetric flask, sonicated and filtered. The filtrate was diluted as per the requirement and 20 µL solution of each of marketed formulations (ZOLINOX® and AMBIEN®) was injected in to the HPLC system for conducting the assay.

Forced degradation studies

Forced degradation studies were performed to evaluate the stability indicating properties and specificity of the method[35]. All solutions for stress studies were prepared at an initial concentration of 1.0 mg/mL of Zolpidem tartrate and refluxed for 30 min at 80 ºC and then diluted with mobile phase. 1.0 mg/mL Zolpidem tartrate solution was exposed to acidic degradation with 0.1 M HCl for 30 min at 80 ºC the stressed sample was cooled, neutralized and diluted with mobile phase. Similarly stress studies were conducted in alkaline conditions with 0.01 M NaOH at 80 ºC for 30 min. and neutralized after cooling with proper dilution with mobile phase. Oxidative stress studies were performed using 30 % H_2O_2 and thermal stress studies were conducted in thermostat at 80 ºC for 30 min. 20 µL solution of each of these solutions which were exposed to forced degradation studies were injected in to the HPLC system and the chromatograms were recorded.

Results and Discussion

Initially the stressed samples were analyzed using a mixture of water: methanol (50:50, v/v) with a flow rate of 1.0 mL/min in which the peak symmetry was not satisfactory. The mobile composition was modified as 30: 70, v/v with flow rate 1.2 mL/min where a broad peak was eluted with slight tailing. Finally the mobile phase composition was modified as water: methanol: acetic acid (25:75: 0.1, v/v) with flow rate 1.2 mL/min and a sharp peak was eluted at retention time 4.20±0.03 min. (UV detection at 254 nm) which was chosen as the best chromatographic response for the entire study.

Zolpidem tartrate shows linearity over a concentration range 0.5-200 µg/mL (Table 1) with % RSD 0.15-0.52. The linear regression equation was found to be y=65834x+8162 (r^2 = 0.9999) (Figure 2). The LOQ was found to be 0.0729 µg/mL and the LOD was found to be 0.0221 µg/mL.

Table 1. Linearity of Zolpidem tartrate

Conc. µg/mL	*Mean peak area ± SD	RSD, %
0.5	32904±78.97	0.24
1	65241±234.87	0.36
5	327081±490.62	0.15
10	664485±2990.18	0.45
20	1326192±2121.91	0.16
50	3359805±17470.99	0.52
100	6527891±18930.88	0.29
150	9973449±30917.69	0.31
200	13124326±55122.17	0.42

Mean of three replicates

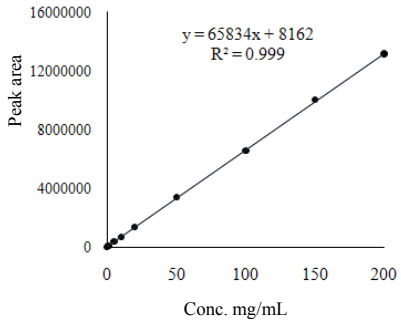


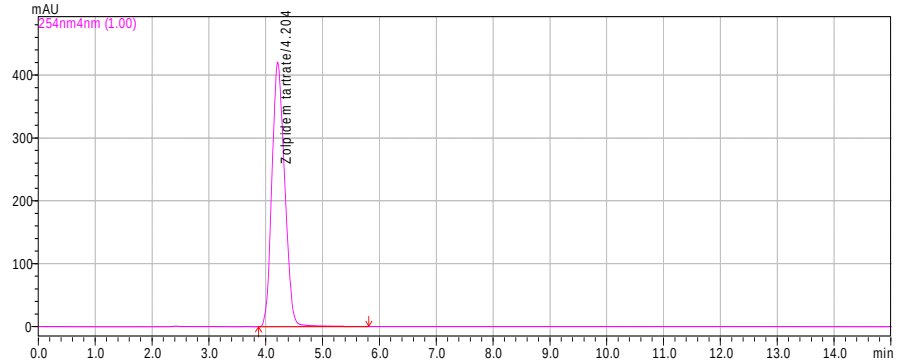
Figure 2. Calibration curve of Zolpidem tartrate

The representative chromatogram of Zolpidem tartrate was shown in Figure 3A. The proposed method was applied to the formulations and the percentage recovery was calculated as 99.74-99.81 (Table 2) without the interference from the excipients (Figure 3B and 3C).

Table 2. Analysis of Zolpidem tartrate commercial formulation (Tablets)

Formulation	Labeled claim mg	*Amount found mg	*Recovery %
Zolinox	7.5	7.48	99.74
Ambien	10	9.98	99.81

Mean of three replicates



[A]

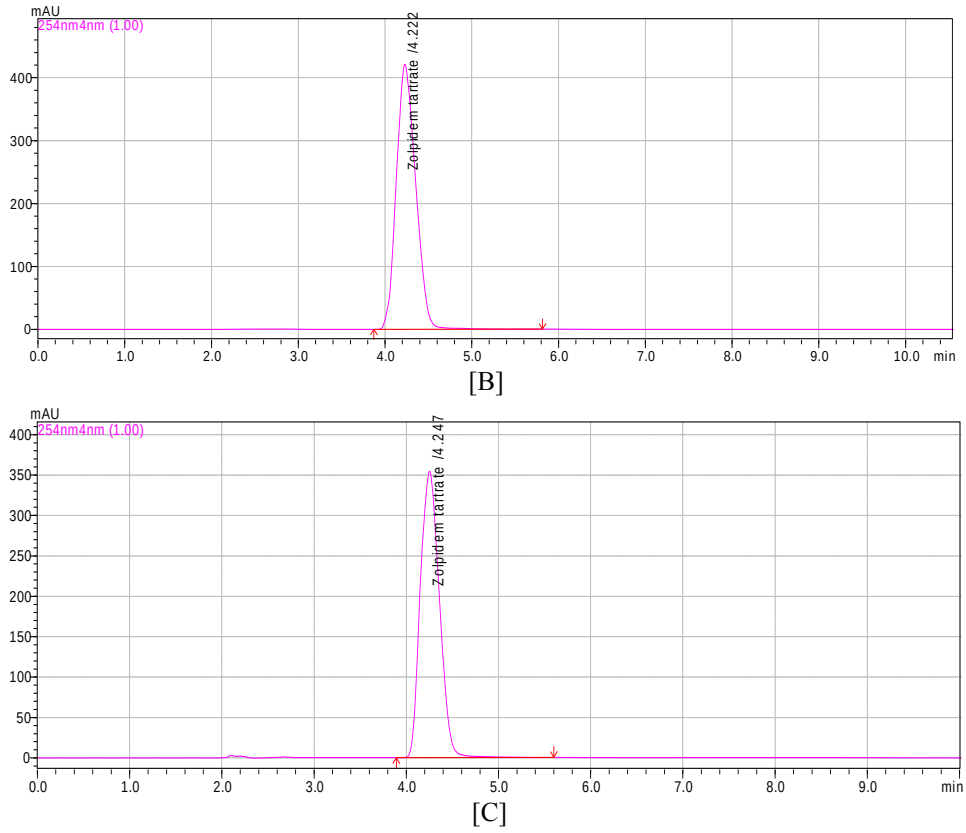


Figure 3. Typical chromatograms of Zolpidem tartrate (100 µg/mL) [A], ZOLINOX (Label claim: 7.5 mg) [B]AMBIEN (Label claim: 10 mg) [C]

The % RSD in precision studies was found to be 0.10-0.37 (Intra-day) and 0.12-0.63 (Inter-day) where as in accuracy studies it is 0.12-0.25 with a recovery of 99.17-99.72 (Table 3) indicating that the method is precise and accurate (% RSD < 2.0). The % RSD was less than 2.0% (0.10-0.35) indicating that the proposed method is robust (Table 4).

Table 3. Precision and accuracy studies of Zolpidem tartrate

Conc. µg/mL	Intra-day precision *Mean peak area ± SD (%RSD)	Inter-day precision *Mean peak area ± SD (% RSD)
20	1330318.00±4896.38 (0.37)	1316677.33±8263.69 (0.63)
50	3359703.33±3257.34 (0.10)	3350140.00±8423.93 (0.25)
100	6527376.00±11249.45 (0.17)	6519172.00±7631.52 (0.12)

Accuracy

Spiked conc. µg/mL	Total conc. µg/mL	*Mean peak area ± SD (% RSD)	Drug found µg/mL	% Recovery
8 (80%)	18	1185487.00±2953.58 (0.25)	17.88	99.35
10 (100%)	20	1313854.33±1588.65 (0.12)	19.83	99.17
12 (120%)	22	1452386.00±2580.90 (0.18)	21.94	99.72

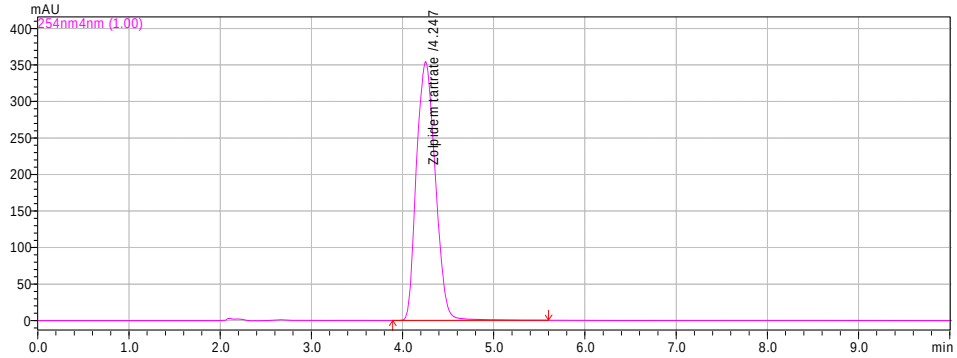
**Mean of three replicates*

Table 4. Robustness study of Zolpidem tartrate

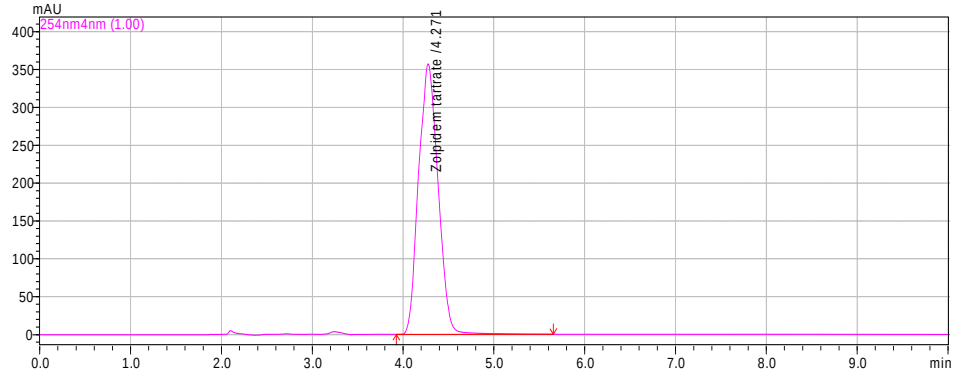
Parameter	Condition	*Mean peak area	*Mean peak area ± SD (% RSD)	*Assay %
Flow rate (± 0.1 mL/min)	1.1	6535288	6525191.33±11682.83 (0.18)	99.96
	1.2	6527891		
	1.3	6512395		
Detection wavelength (± 2 nm)	253	6502525	6521626.00±16887.93 (0. 26)	99.90
	254	6527891		
	256	6534522		
Mobile phase composition (± 2 %, v/v)	23: 77	6521590	6521501.00±6434.96 (0.10)	99.90
	25: 75	6527891		
	27: 73	6515022		

**Mean of three replicates*

The stability indicating capability of the method was established from the separation of Zolpidem tartrate peak from the degraded samples. Zolpidem tartrate has shown 22.03% and 25.35% degradation during acidic and oxidative stress conditions indicating that the drug is sensitive where as in other degradations the drug has undergone decomposition slightly (< 20.0%) (Table 5). Typical chromatograms obtained from the stressed samples were shown in Figure 4A-4E.



[A]



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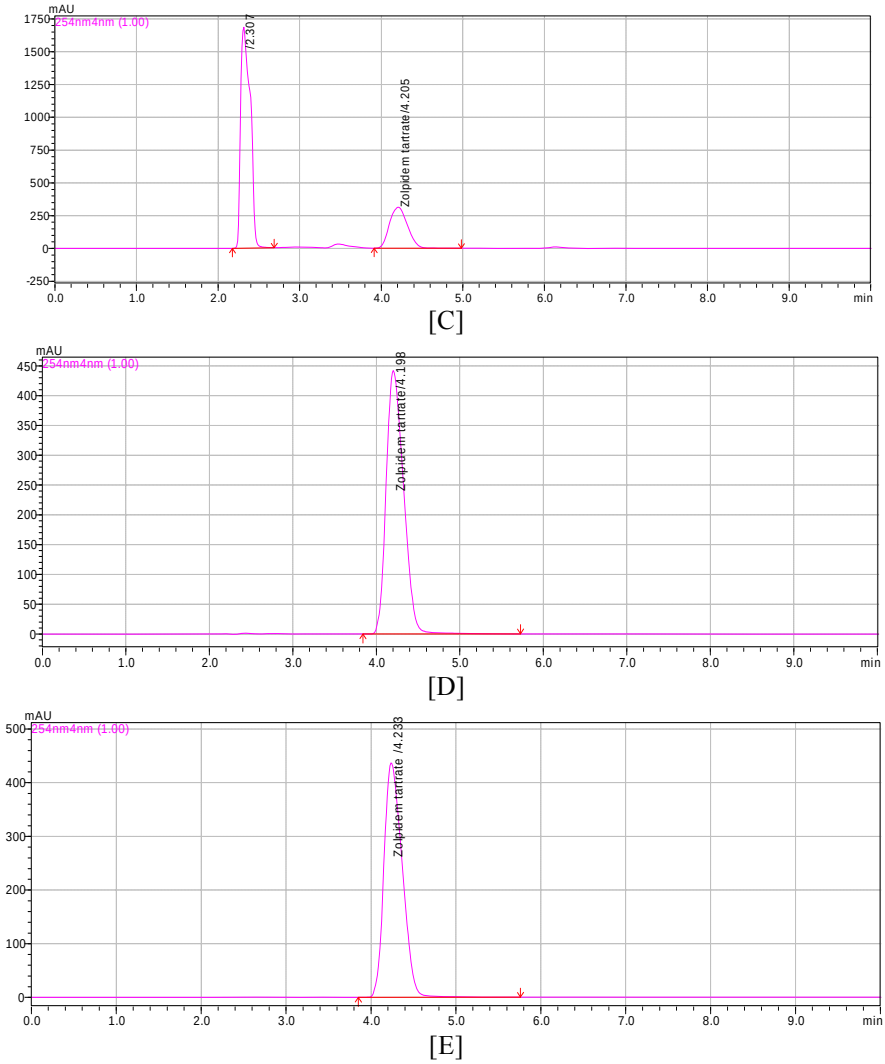

Figure 4. Typical chromatograms of Zolpidem tartrateon acidic [A] alkaline [B] oxidative [C] thermal [D] photolytic [E] degradation

Table 5. Forced degradation studies of Zolpidem tartrate

Stress Conditions	*Mean peak area	*Drug recovered %	*Drug decomposed %
Standard drug (Untreated)	6545101	100	-
Acidic degradation	5103406	77.97	22.03
Alkaline degradation	5341037	81.60	18.40
Oxidative degradation	4886007	74.65	25.35
Thermal degradation	6378709	97.46	2.54
Photolytic degradation	6533255	99.82	0.18

**Mean of three replicates*

The present stability-indicating method for the determination of Zolpidem tartrate in pharmaceutical formulations is specific because the drug peak was well separated even in the presence of degradation products. The system suitability parameters for the Zolpidem tartrate peak shows that the theoretical plates were more than 2000 and the tailing factor was less than 2 (or <1.5-2.0).

Conclusion

The proposed stability-indicating HPLC method was validated as per ICH guidelines and can be successfully applied to perform long-term and accelerated stability studies of Zolpidem tartrate formulations.

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