

Base Hydrolytic Forced Degradation Study of Zolpidem Tartrate by HPLC

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(Received September 27, 2011; Revised December 25, 2012; Accepted December 30, 2012)

Abstract: Zolpidem was subjected to ICH prescribed base hydrolytic stress study. The stability indicating assay was established by using isocratic RP-HPLC separation C18 column (waters Spherisorb 250mm length×4.6 mm internal diameter and 5 µm particle size) for degradant of zolpidem by base hydrolysis. The mobile phase comprising of methanol: water (900:100, v/v) was used. The flow rate was adjusted to 1.0 mL/min and detection was performed at 254 nm using a PDA detector. The impurity was characterized by mass spectral studies. The degradant appeared at relative retention time (RRT) of 0.3939 min of zolpidem tartrate. The validation studies established a linear response of 0.0025-0.0125 mg mL⁻¹ for the drug and satisfactory results for precision and recovery studies without interference with detection of zolpidem tartrate. Limits of detection and quantification (LOD and LOQ) were 150 and 450 ng, respectively for zolpidem tartrate. The MS studies for probable structural confirmation show m/z value of the peak as 279. The pure impurity was used for correlation with the degradant formed. A simple, precise, and accurate isocratic reversed-phase stability-indicating high performance liquid chromatographic assay method was developed and validated for determination of zolpidem tartrate.

Keywords: Forced decomposition; degradant; base hydrolysis; Zolpidem tartrate; validation

1. Introduction

Zolpidem is a prescription medication used for the short-term treatment of insomnia, as well as some brain disorders. It is a rapid acting hypnotic agent of relatively short duration of action [1,2].

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The chemical name for zolpidem tartrate is (N, N, 6-trimethyl-2-(4-methylphenyl)-imidazo (1, 2-a) pyridine-3-acetamide) (ZOLP). The empirical formula for ZOLP is C₁₉H₂₁N₃O HCl. It is a short-acting non-benzodiazepine hypnotic that potentiates gamma-amino butyric acid (GABA), an inhibitory neurotransmitter, by binding to benzodiazepine receptors which are located on the gamma-amino butyric acid receptors. It is being formulated as 2:1 tartrate salt.

To the date, there is no publication concerning the stability-indicating analytical method with base hydrolysis for ZOLP. Only one stress condition that is acid hydrolysis by using 30 mL of 2M HCl refluxed for 2 hours is reported [3]. However, several methods have been reported for determination of the compound in bulk powder and pharmaceutical preparations. Forensic biological samples studied by electrophoresis [4], radio immune assay [5], voltametry [6], liquid chromatography [7], potentiometry [8], LC-MS-MS [9], GC-NPD [10].

Once a product is designed and developed, the stability of a bulk product is required to be demonstrated for the “shelf-life” of a product before the formulation. Shelf life refers to the time for which the drug product retains the quality specifications. Depending upon the clinical consequence, the agency might tighten the potency/strength requirement. The estimated/tentative shelf-life is backed by real time forced degradation studies at controlled conditions.

Thorough pre-formulation work is the foundation of developing robust formulations. Chemical stability by means of base hydrolytic degradation mechanism is one of the important of other mechanisms.

Forced degradation studies are designed to generate assurance of safety (degradant levels) i.e. for toxicological quantification of degradants at anticipated maximum level of exposure and product-related variants and develop analytical methods to determine the degradation products formed during accelerated pharmaceutical studies and long-term stability studies. Any significant degradation product should be evaluated for potential hazard and the need for characterization and quantitation.

The forced degradation studies are also expected to elucidate possible degradation path-ways, to identify degradation products that may be spontaneously generated during drug storage and use to facilitate improvements in the manufacturing process and formulations in parallel with accelerated pharmaceutical studies. Stress studies may be useful in determining whether accidental exposures to conditions other than normal ranges (e.g., during transportation) are deleterious to the product, and also for evaluating which specific test parameters may be the best indicators of product stability.

Stress testing is a critical component of drug development. By generating key stress-testing samples (i.e., partially degraded samples stressed under various conditions), predictive degradation information can be obtained early in the process and can be of significant value to a drug company in terms of time and money. In addition, stress testing can help in the selection of more-stable drug substance salt forms and drug formulations [11].

The International Conference on Harmonization (ICH) guidelines [12] requires performing stress testing of the drug substance, which can help identify the likely degradation products and can be useful in establishing degradation pathways and validating the stability-indicating power of the analytical procedures used. Moreover, validated stability indicating method should be applied in the stability study.

The objective of the present paper was to develop a validated stability-indicating HPLC assay for determination of ZOLP thereby to test the sensitivity of ZOLP towards base hydrolysis and mass characterization of the base hydrolyzed product. The impurity originated is not only a degradation impurity but also one of the process related impurity [13]. Method validation was done according to ICH guidelines.

2. Materials and Methods

2.1. Materials

Standard reference material (SRM) of ZOLP and its impurity standard namely Zolpacid was received as gift samples from Process Research Department of Integrated product development operations of Dr. Reddy's Laboratories Limited, Hyderabad, India. HPLC grade acetonitrile, methanol, were purchased from Merck, Germany. High pure water was prepared by using Millipore Milli Q plus purification system.

2.2. Instrumentation

High Performance Liquid Chromatography (HPLC) system used Waters make instrument consisting of a 600E controller pump, a 717 plus auto sampler, 2996 PDA detector and an inline-degasser. For data processing Millinium32 software was used. The chromatographic separation was achieved on a C18 Waters Spherisorb, 250 mm x 4.6 mm, 5 μm column, using a mobile phase containing mixture methanol and water (900:100, v/v). The mobile phase was filtered through a nylon membrane filter (pore size 0.45 μm) and degassed and filtered through 0.45 μm nylon membrane filter. Ultrasonicator was used for sonication. Shimadzu analytical balance was used for weighing. A 1mlh remi magnetic stirrer was used to carry out Base hydrolysis. The flow rate of the mobile phase was kept at 1.0 mL/min. The HPLC column was maintained at ambient temperature and the wavelength was monitored at 254 nm. The injection volume was 10 μL . Methanol was used as diluent during the standard and test samples preparation. Mass spectrometric characterization was performed on Perkin-Elmer Clarus 600S GC-MS system controlled by Turbomass 5.3.0 software. Electron-impact (EI) ion source (electron energy 70 eV) and an electron multiplier detector capable of recording ions from m/z 26 to m/z 400. The capillary column was a 15 m x 0.25 mm i.d. fused-silica column coated with a 0.1 μm film of Elite-5MS. Injector temperature 260 $^{\circ}\text{C}$, inline and source temperature was kept at 150 $^{\circ}\text{C}$. The column oven temperature was initially maintained at 90 $^{\circ}\text{C}$ for 0.7min then programmed @ 35 $^{\circ}\text{C}$ min⁻¹ to 240 $^{\circ}\text{C}$ which was further increased @ 8 $^{\circ}\text{C}$ min⁻¹ to 290 $^{\circ}\text{C}$ which was held for 6 minutes which was further increased @ 25 $^{\circ}\text{C}$ min⁻¹ to 325 $^{\circ}\text{C}$. Solvent delay of initial 2 minutes.

2.3. Conduct of Stress study

Base decomposition was carried out in 5N NaOH at drug strength of 0.5 mg/mL. As sufficient decomposition was not observed, the drug was additionally refluxed at a concentration of 1 mg/mL in 5 N NaOH at 50 $^{\circ}\text{C}$ for 6 hrs with 500 rpm speed of magnetic stirrer.

2.4. Separation studies

Before injecting to HPLC, the volume of reaction solution was made to 10 mL with methanol. The study was conducted using a mobile phase composed of methanol: water [900:100, v/v]. The separation was achieved by changing the mobile phase composition, diluent, as well as the flow rate.

2.5. Validation of the developed stability-indicating method

Validation of the optimized HPLC method was done with respect to various parameters, as required under ICH guideline Q2 (R1) [14]. A stock solution of ZOLP (200 µg/mL) was prepared by dissolving appropriate amount in the diluent. Working solutions of 0.8, 1.6, 2.4, 3.2 and 4 µg/10 mL were prepared from above stock solution for related substances determination. A stock solution of zolpacid impurity at 200 µg/mL was also prepared in diluent.

To establish linearity and range, a stock solution of the drug was prepared at strength of 0.5 mg/mL, which was further diluted to prepare solutions in the drug concentration range of 0.25-1.25 µg/mL. The solutions were injected in triplicate into the HPLC column, keeping the injection volume constant (10 µL). Precision of the method was studied by making six injections of three different concentrations, viz., 0.25, 0.75 and 1.25 µg/mL from concentration range 0.25- 1.25µg/mL. The precision studied on the same day and the values of relative standard deviation (% RSD) were calculated to determine intra-day precision. These studies were also repeated on different days to determine inter-day precision. Accuracy was evaluated by fortifying a mixture of degraded solutions with known concentration of, viz., 100 µg/mL of drug for degraded solution of base hydrolysis. The recovery of the added drug was determined. The specificity of the developed HPLC method for ZOLP was carried out in the presence of its impurity, Zolpacid.

2.6. Characterization of degradation product

Mass spectrometric study was carried out to determine the m/z value of the degradant formed under base hydrolytic condition. The obtained value was compared with the molecular weight of probable degradant by base hydrolysis of Zolpidem tartrate.

2.7. Co-injection of impurity

The conformational study was also done by injecting standard zolpacid impurity in to the chromatographic system. The retention time (RT) of the standard zolpacid impurity and the impurity formed in base hydrolyzed solution was same.

3. Results and Discussion

RT and relative retention time (RRT) of the drug and the degradation product is listed in Table 1. The data obtained from linearity study is given in Table 2.

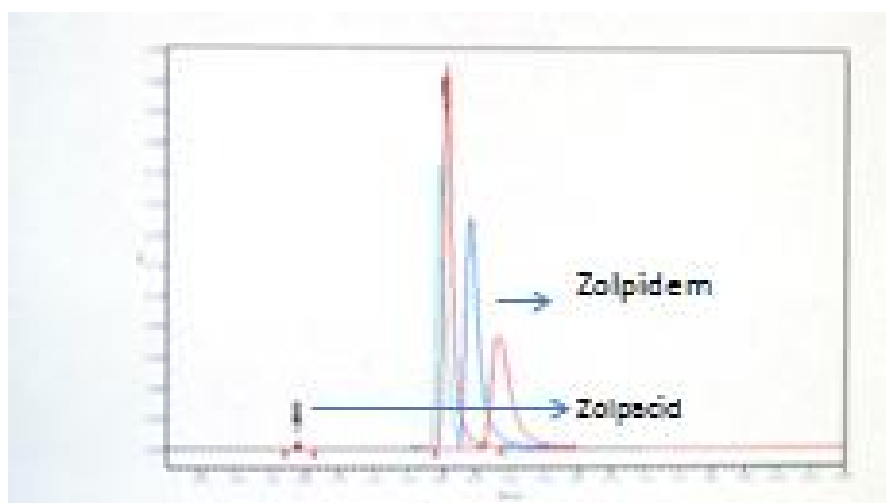
Table 1. Retention time and relative retention time of degradation peak

Peak	Retention time (RT)	Relative retention time (RRT)
Zolpidem tartrate	4.63	1.00
Zolpacid	1.82	0.393

Table 2. Linearity data on three different days

Regression parameter	Day 1	Day 2	Day 3	Mean±SD(%RSD)
Slope	4.328	4.326	4.330	4.328±0.002(0.05)
Intercept	-0.102	-0.101	-0.104	-0.1023±0.001(-1.49)
R ²	0.9992	0.9994	0.9990	0.9992±0.0002(0.02)

The average % recovery of the drug was observed 99.84. The precision data is given in Table 3. Good separation was achieved even when the method was repeated by a different analyst, thus confirming the reproducibility of the method. As shown from the data in Table 4, good recoveries were made at added concentrations of 100 µg/mL of drug for degradation solution of base hydrolysis. The average % recovery of the drug was observed 99.84 from degradation solutions of base hydrolysis.

**Figure 1.** Chromatogram showing recovery study of zolpidem in presence of base hydrolysis degradant, zolpacid. zolpidem tartrate (RT 4.63 min): Zolpacid (RT 1.82 min)**Table 3.** Reproducibility and precision data obtained during intra-day (n=6), inter-day (n=3) studies.

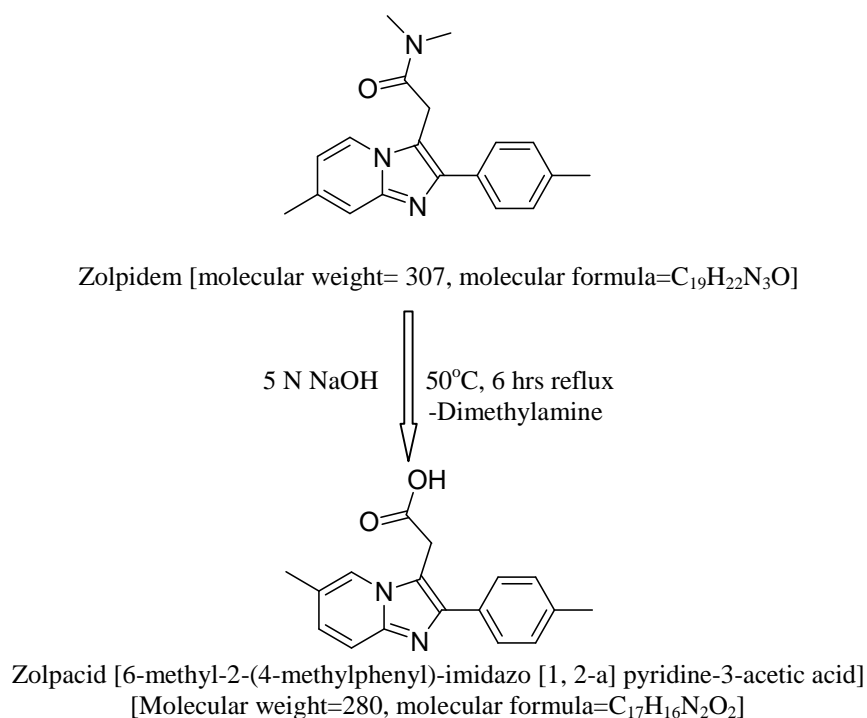
Actual Concentration (µg/mL)	Intra-daymeasured concentration (µg/mL) (% RSD)	Inter-daymeasured concentration (µg/mL) (% RSD)
0.25	0.248(0.41)	0.236(3.23)
0.75	0.733(1.91)	0.795(3.50)
1.25	1.252(0.40)	1.254(0.21)

Table 4. Recovery studies using different dilutions from degradation solution (n=6)

Volume of acid degradation solution diluted to 10 mL	Actual added pure concentration ($\mu\text{g/mL}$)	Measured of pure drug concentration ($\mu\text{g/mL}$)	Recovery (%) (n=6)
0.2	200	197.6	98.80
0.6	200	203.06	101.53
1.0	200	198.4	99.20
		Average recovery	99.84

3.1. Degradation behavior

In total, a single degradant was detected by HPLC on decomposition of the drug under basic condition and it was characterized by mass spectrometry. The degradation behavior of the drug in base hydrolytic stress condition is outlined below (Figure 2).

**Figure 2.** Suggested mechanism for the base hydrolysis of zolpidem.

Base hydrolysis: The drug degraded on heating at 50°C for 6 hrs in 5N NaOH, forming single degradation peak at RRT 0.3939. The reaction in 5N NaOH at room temperature for 1 hr did not result in significant degradation.

3.2. Development and optimization of the stability-indicating method

The resolution of degradant from the drug was influenced by composition of mobile phase. No good separation seen when only methanol was used as mobile phase. The acceptable separation with reasonable peak shapes and peak purity was achieved by using mobile phase comprising of methanol:water [900:100, v/v]. Both ZOLP and zolpacid impurity peaks got split when mobile phase was used as diluent for sample preparation. Then, pure methanol was used as diluent for sample preparations.

3.3. Validation of the developed stability-indicating method

The validation studies established a linear response of the drug at concentrations between 0.25-1.25 µg/mL in mobile phase methanol:water (900:100,v/v). LOD and LOQ values were 150 ng and 450 ng respectively

3.4. LOD and LOQ of impurity

The limit of detection and limit of quantification of zolpacid impurity was found to be 20 ng and 60 ng, respectively.

3.5. Characterization of degradation product

Mass chromatogram in the positive electron spray (ESI) mode for the degradant is shown in Figure 3. The m/z value of the peak is 279 which matched 6-methyl-2-(4-methylphenyl)-imidazo [1, 2-a] pyridine-3-acetic acid as degradant of ZOLP by base hydrolysis. The observed fragmentation pattern was taken into consideration for confirmation of chemical structure.

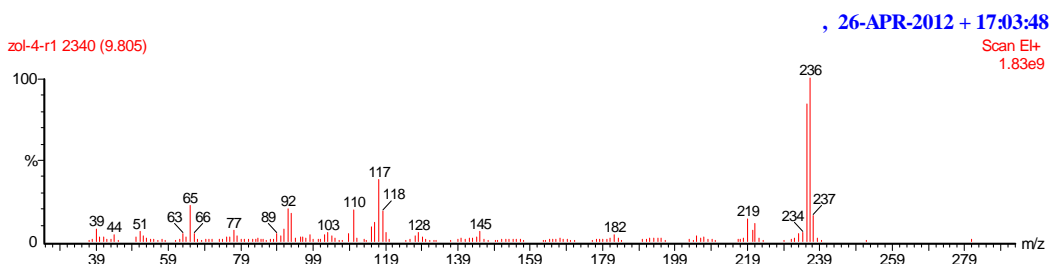


Figure 3. Mass spectra of peak 1(zolpacid, RT=1.82)

4. Conclusion

It was possible in this study to develop a stability-indicating HPLC assay method for ZOLP by subjecting the drug to ICH recommended stress condition. The drug and degradant got well separated

from each other in an isocratic mode using a reversed-phase C18 column in mobile phase comprising of methanol:water [900:100]. The method proved to be simple, accurate, precise, specific and selective. The stress studies and subsequent MS analyses showed that the drug was decomposed to degradation product, 6-methyl-2-(4-methylphenyl)-imidazo [1,2-a]pyridine-3-acetic acid.

Supporting Information

Supporting information accompanies this paper on <http://www.acgpubs.org/JCM>

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