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Rapid, Simple and Validated Method for Estimation of Zolpidem by UPLC in Tablet Dosage Form

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Authors' contributions

This work was carried out in collaboration between both authors. Author RAP reviewed the various literatures, prepared the necessary sample and standard solutions, performed the statistical analysis and managed the analyses of the study. Author VRJ designed the method, wrote the first draft of manuscript and evaluated validation parameters. Both authors read and approved the final manuscript.

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ABSTRACT

Aim: To create and approve a novel and fast RP-UPLC technique for the estimation of zolpidem in tablet dosage form.
Place and Duration of Study: Department of pharmaceutical analysis, S.V.S. school of Pharmacy, Bheemaram, between January 2013 and May 2013.
Methodology: Chromatographic division was accomplished on a Waters Acquity HSS T-3 C_{18} stationary phase (100 × 2.1 mm, 1.8 µm) utilizing an isocratic technique with versatile mobile phase made out of Potassium di-hydrogen phosphate: Acetonitrile in the proportion 40:60 v/v at a stream rate of 1 mL/min. The temperature was kept constant and detection was made at 243 nm. The run time was as short as 6 min. The proposed method was validated according to the International Conference on Harmonization (ICH) rules for parameters such as linearity, precision, accuracy, specificity and robustness etc.

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Results: The developed method was linear for zolpidem from 30-70 µg/ml and the linear regression obtained was > 0.999. Precision, established by intra- and inter-day assays shown relative standard deviation (R.S.D) values within 1.5%. Recovery data were in the range 99.8% to 101.4% with R.S.D. values < 1.5%.
Conclusion: The proposed method satisfied all the validation parameters prescribed by ICH. The ultra fast analysis with RT 2.754 min renders the analysis of a bulk samples in a less time and, therefore, should be cost effective for everyday analysis in the estimation of Zolpidem.

Keywords: Zolpidem; UPLC; new method development; validation.

1. INTRODUCTION

In this requirement for speed period the analytical research centres are searching for better approaches to cut expenses and abbreviate time for logical strategy for QC of dosage forms while in the meantime enhancing the affectability and selectivity of the technique. In spite of the fact that elite liquidchromatography (HPLC) is a settled dependable procedure utilized as a part of controlling the quality and consistency of dynamic pharmaceutical fixings (API's) and dose shapes, it is frequently a moderate method as a result of the multifaceted nature of a portion of the specimens, it could even now be made strides. Another class of partition strategy, UPLC, has turned out to be a standout amongst the most encouraging advancements in the zone of quick chromatographic separation with its one of a kind attributes of high chromatographic determination, speed, and affectability examination. UPLC, by Using sub-2 µm particles and versatile stages at high straight speeds, and instrumentation that works at higher weights than those utilized as a part of HPLC, favours the drastic increment in determination, affectability, and speed of investigation. In this way, by diminishing the molecule size of the stationary phase, more noteworthy determination is accomplished between crests, or a similar determination can be accomplished in less time. Likewise, more noteworthy section productivity brings about smaller pinnacles, expanded analyte determination, and, accordingly, the enhanced analyte detection. The fundamental favourable position of diminishing molecule estimate is that it offers better determination as the surface territory increments. Sub 2-µm particles show an extra advantage in that they keep up productivity of division over a significantly more extensive straight speed than bigger particles. Investigation of working expense and test throughput discovered UPLC cost beneficial over HPLC. In the present work, this innovation has been connected to the strategy improvement and approval investigation of related substance and test assurance of zolpidem. In the present work, this technology has been applied to the method development and validation study and assay determination of zolpidem [1,2].

Zolpidem chemically is a N, N-dimethyl-2-(6-methyl-2-p-tolylimidazo [1,2a] pyridin3yl) acetamide (Fig. 1). Zolpidem, an imidazopyridine derivative, is a hypnotic indicated for the temporary treatment of insomnia. Zolpidem enhances rest patients with sleep deprivation.

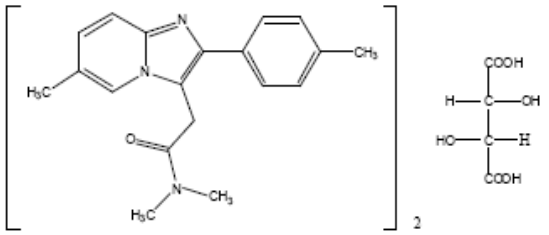

Fig. 1. Structure of zolipidem hemitartrate

The free base has a molecular weight of 307.4, with the salt form of having a formula weight of 764.9, with a melting point of 193-197℃. The tartrate salt is highly soluble in water, sparingly soluble in methanol, and practically insoluble in methylene [3,4].

The literature survey provides some of the references for the estimation of Zolpidem, by HPLC [5-11], LC/MS [12], GC/MS [13,14], LC/MS/MS [15-17] and radioimmunoassay [18]. As LC/MS, GC/MS and GC techniques are expensive and not affordable in quality control laboratories. As LC/MS, GC/MS and GC procedures are not financially savvy and not routinely moderate in quality control research centers. The announced HPLC techniques are additional tedious, complex versatile stage blends, utilize high stream rate of examination, absence of affectability and pinnacle symmetry. However there were no reports accessible on the estimation of Zolpidem by UPLC strategy. It is, in

this way, felt important to build up another quick technique for the assurance of Zolpidem by UPLC strategy. Consequently a reproducible RP UPLC strategy was produced for the quantitative assurance of Zolpidem tablets by utilizing Waters Acquity HSS T-3 C18 segment (100 × 2.1 mm, 1.8 µm) UPLC column. The proposed strategy was approved according to the rules recommended by ICH [19].

2. EXPERIMENTAL DETAILS

2.1 Materials and Reagents

Zolpidem hemitartrate Working Standard was obtained from Natco research centres, Hyderabad, India. Zolpidem tablets bought from neighbourhood drug store. Acetonitrile HPLC Grade and Ortho phosphoric corrosive AR grade were purchased from Merck chemicals, Mumbai. Water was procured by utilizing Millipore Milli Q Plus water framework.

2.2 Chromatographic Conditions

Chromatography division was performed on Waters Aquity UPLC with UV indicator. The yield flag was checked and handled utilizing enables programming. Waters Acquity HSS T-3 C18 (100 × 2.1 mm, 1.8 µm) utilized as the chromatographic column. The versatile period of Potassium di-hydrogen phosphate: Acetonitrile in the proportion 40:60 v/v at a stream rate of 1 ml/min was chosen as an enhanced condition. The detection was observed at the Wavelength of 243 nm. The infusion volume was 20.0 µL and the chromatographic runtime of 6 min was choosen.

2.3 Preparation of Solutions

2.3.1 Preparation of phosphate buffer

Accurately weighed 7.0 grams of Potassium di hydrogen phosphate and transferred into a 1000 ml beaker and diluted to 1000 ml with milli pore water. Adjusted the pH to 4.0 with ortho phosphoric acid.

2.3.2 Preparation of mobile phase

Mix a mixture of above buffer 400 mL (40%) and 600 mL of methanol (60%) and degas in ultrasonic water bath for 5 minutes. Filter through 0.45 µ filter under vacuum filtration.

2.4 Preparation of the Zolpidem Hemitartrate Standard and Sample Solution

2.4.1 Standard solution preparation

Accurately transferred 10 mg of zolpidem hemitartrate working standard into a 10 mL volumetric flask and about 7 mL of diluents added then sonicated to dissolve it completely and the volume was made up to the mark with the same solvent (Stock solution). Further pipette 0.5 ml of the above stock solution into a 10 mL volumetric flask and diluted up to the mark with diluents. Mix well and filter through 0.45 µm filter.

2.4.2 Sample solution preparation

Accurately transferred the sample equivalent to 10 mg of zolpidem hemitartrate into a 10 mL volumetric flask. About 7 mL of diluent added and sonicated to dissolve it completely and the volume is made up to the mark with diluent. Mixed well and filtered through 0.45 µm filter. Further pipetted 5 ml of the above stock solution into a 50 mL volumetric flask and diluted up to the mark with diluent. Mix well and filter through 0.45 µm filter. Further pipetted 3 ml of the above stock solution into a 10 ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45 µm filter.

2.5 Method Validation

2.5.1 Precision

The precision of the method was estimated by taking out six independent asses of test sample against a qualified reference standard and the %RSD of assay was calculated (% RSD should not be more than 2%).

2.5.2 Intermediate precision/Ruggedness

2.5.2.1 Intra-day precision

The precision of the assay method tested by taking five independent assays of Zolpidem hemitartrate (50,100, 150% i.e. 5.0, 10.0, 15.0 µg/ml.) test samples against qualified reference standard. The percentage of RSD of five assay values was calculated.

2.5.2.2 Intermediate precision (inter-day)

Different analyst from the same laboratory and by using different column of same brand were

used to evaluate the intermediate precision of the method. This was performed by assaying the six samples of Zolpidem hemitartrate against qualified reference standard. The percentage of RSD of six assay values was calculated. The %RSD for the area of six replicate injections was found to be within the specified limits (% RSD should not be more than 2%).

2.5.3 Accuracy

Recovery of the assay method for Zolpidem hemitartrate was evaluated by three determinations of test sample using tablets at 50%, 100% and 150% of analyte concentration. Every sample was infused thrice (n=3) into UPLC framework and the normal pinnacle range was computed from which Percentage recuperations were figured. (% Recovery ought to be between 98.0 to 102.0%).

2.5.4 Linearity

Test solutions were prepared from stock solution at 5 concentration levels (30, 40, 50, 60 and 70 µm/ml). The peak area vs. concentration data treated by least square linear regression analysis (Correlation coefficient ought to be not less than 0.999).

2.5.5 Limit of detection (LOD) limit of quantification (LOQ)

LOD and LOQ for the were determined at signal to noise ratios of 3:1 and 10:1, respectively by injecting series of dilute solutions with known concentrations.

2.5.6 Robustness

To demonstrate the unwavering quality of the investigative technique amid ordinary use, some little yet ponder changes were made in the analytical method (e.g., flow rate, column temperature, and mobile phase composition). Changes in the chromatographic parameters (i.e., theoretical plates and the tailing factor) were assessed for the studies.

3. RESULTS

3.1 Method Development

Distinctive chromatographic conditions were tested to accomplish better productivity of the chromatographic system. Modifications such as mobile phase composition, wavelength of detection, column, column temperature, pH of mobile phase, and diluents were optimized. Several ratios of buffer, and solvents (water, methanol and acetonitrile) were checked in order to obtain optimum composition of the mobile phase. Retention time, tailing factor, theoretical plates, flow rate and run time were the major considerations while developing the method. Acquity BEH C_{18}, 50 mm × 2.1 mm, 1.7 µm column used for the elution, but the peak eluted before 4.5 minutes with a tailing factor of 2. Experiment with Phenyl 100 mm × 2.1 mm, 2 µm column ended with inconsistent retention time and peak fronting. Buffers like sodium dihydrogen orthophosphate, dipotassium hydrogen orthophosphate, and disodium hydrogen orthophosphate did not yield required results. Use of ion pair reagents also did not yield the the normal pinnacle.

At 40:60 (buffer: solvent) ratio of the mobile phase, a perfect peak was eluted. Thus the mobile phase ratio was fixed at 40:60 (buffer: solvent) in an isocratic mobile phase flow rate. The typical chromatogram obtained for Zolpidem from final UPLC conditions are shown in Fig. 2.

3.2 Method Validation

In view of International Conference on Harmonization (ICH) rules, the technique is approved with respect to system suitability, linearity, accuracy, precision, LOD, LOQ, robustness and sensitivity as follows.

3.2.1 System suitability

The system suitability results for the proposed UPLC method are Tailing factor Obtained from the standard injection is 1.6. Theoretical Plates obtained from the standard injection is 8792.3. The outcomes demonstrated that the streamlined UPLC strategy satisfies these necessities within the USP accepted limits indicated in the 'Experimental' section.

3.2.2 Precision

The % R.S.D. of Zolpidem assay during the method precision was found to be 0.73%, indicating good precision of the method. The results are summarized in Table 1.

3.2.3 Intermediate Precision

The % R.S.D. of Zolpidem assay during the intermediate precision was found to be 0.728%

the value is well within the generally acceptable limits. The results are summarized in Table 2.

Table 1. Results of precision

Injection	Area
Injection-1	4796667
Injection-2	4712916
Injection-3	4721422
Injection-4	4771493
Injection-5	4750737
Average	4750647
Standard Deviation	34749.6
%RSD	0.73%

3.2.4 Limits of detection (LOD) and quantification (LOQ)

LOD and LOQ for Zolpidem were 0.01 and 0.05µg/ml, respectively. Since the LOQ and LOD values of Zolpidem are achieved at a very low level, this method can be suitable for cleaning validation in the pharmaceutical industry.

3.2.5 Accuracy

Percentage recovery of Zolpidem samples ranged from 99.8% to 101.4% and the mean recovery is 99.8%, showing the good accuracy of the method. The result is shown in Table 2.

3.2.6 Linearity

The linearity of the calibration plot for the method was obtained over the calibration ranges tested, i.e., 30 - 70 µg/ml for three times, and the correlation coefficient obtained was 0.999, thus indicating excellent correlation between peak areas and concentrations of the analyte.

3.2.7 Robustness

In all the deliberately varied chromatographic conditions in the concentration range for the evaluation of robustness is 10 -50 µg/ml, (n=3). It can be concluded that the variation in flow rate and the variation in 10% Organic composition do not affect the method significantly. Hence it indicates that the method is robust even by change in the flow rate ±10% and change in the Mobile phase ±10%.

3.2.8 Application of the developed method to commercial Zolpidem tablets

When the developed method was used to analyze a commercial brand of Zolpidemtablet formulation, the mean recovery of five replicates was 99.69% with % R.S.D. of 0.52. The % recovery value indicates non-interference from the excipients present in the dosage form.

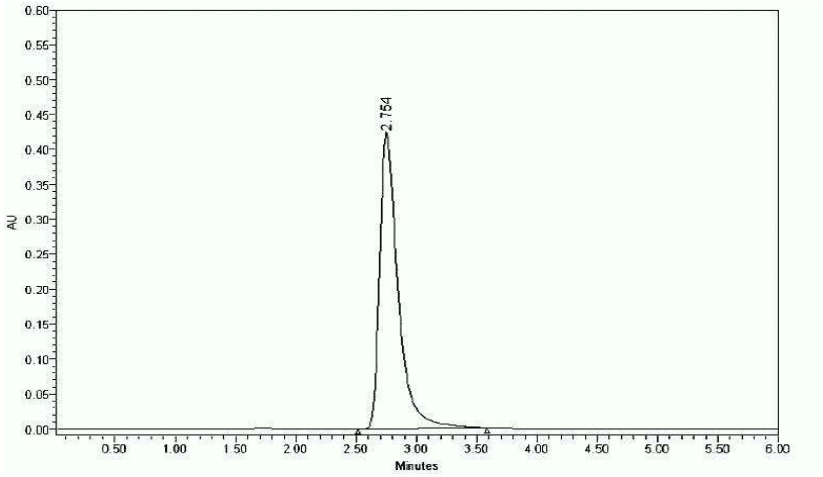

Fig. 2. Typical chromatogram of Zolpidem by proposed method

Table 2. Results of accuracy

%Concentration (at specification level)	Area	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
50%	4838809	5.0	5.07	101.4%	99.8%
100%	9462459	10.0	9.91	99.1%	
150%	14149612	15.0	14.8	98.8%	

4. DISCUSSION

4.1 Method Development and Optimization

The principle point of the developed method is to attain separation and furthermore quantification for Zolpidem utilizing an isocratic versatile RP-UPLC framework. Identification for Zolpidem might have been sufficient at 243 nm. Developing a UPLC method was to reduce the run time of the method and solvent consumption for routine analysis viz assay, dissolution and content uniformity during QC. To avoid any degradation and interference, a mobile phase with pH 4 was selected. The mobile phase was selected in the ratio of Potassium di-hydrogen phosphate: ACN in the ratio 40:60 v/v at a flow rate of 1 ml/min. While developing the UPLC method, basic chromatographic conditions such as the column, solvents and UV detection employed in the HPLC method were taken under record. On selecting the UPLC column, its Strength toward the higher ph might have been thought seriously about to preserve those long an aggregation of the section. Waters Acquity HSS T-3 C18 column (100 × 2.1 mm, 1.8µm) column was found to be more suitable and stable at this pH. The peak was sharp and well resoluted. The flow rate also is brought down from 2.0 to 1 ml/min. When these operating parmeters were applied to the developed method, a satisfactory peak was achieved for Zolpidem, which eluted at around 2.754 min at a total run time of 6 min.

5. CONCLUSION

The proposed RP-UPLC method proved to be simple, linear, precise, accurate, robust and rapid. The developed method has been skilled of giving faster elution, maintaining good separation greater than that achieved with conventional HPLC. The low limit of detection and quantification makes this method superior method to the existing methods. The short retention time of 2.754 min allows the analysis of a large number of samples in a short period of time and is therefore more cost-effective for routine analysis in the quality control laboratories. It is suitable for faster, reliable and accurate quality control of Zolpidem in dosage forms.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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