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Research Article

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Development of stability indicating RP-HPLC method and validation for the estimation of zolpidem tartrate

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ABSTRACT

An accurate, precise, rapid and economical RP-HPLC method has been developed for the estimation of Zolpidem tartrate as per ICH guideline in pharmaceutical dosage from use ultra violet (UV) detector. Elution was carried out using a mobile phase consisting of Methanol, Acetonitrile and Ammonium acetate buffer of which pH was adjusted at 7.4 in a ratio (40:40:20v/v) and flow rate was set on 1ml/min at 254 nm, retention time for Zolpidem tartrate was found to be 5.178 min. The method was found to be linear in the concentration range of 07-13 ug/ml, in the linearity study regression equation was found to be y = 23111.46x and correlation coefficient was found to be 0.9997. This method was Rugged and Robust in different testing criteria, LOD and LOQ was found to be 0.02 µg / ml and 0.05 µg / ml respectively. Accuracy study was done in three different concentration level i.e 70, 100, 130% and % recovery of the method was found to be 99.97%, 100.02%, 100.02% respectively in 3 different levels and mean recovery was 100.00%, so method was accurate. Results of all validation parameter were within the limit as per ICH guideline.

Keywords: Zolpidem tartrate (ZOL), RP-HPLC, Method Development, Validation, Stability Indicating, ICH guidelines

INTRODUCTION

Zolpidem tartrate (ZOL) is a non-benzodiazepine hypnotic of the imidazopyridine class and is available in 5 mg and 10 mg strength tablets for oral administration. Chemically, It is N, N, 6- trimethyl-2-p-tolylimidazo [1,2-a] pyridine-3-acetamide L-(+)-tartrate. (Fig.1) The drug is official in British Pharmacopoeia [1] and Merck Index [2]. It is a white to off-white crystalline powder that is sparingly soluble in water, alcohol, and propylene glycol. It produces agonistic effect on GABA receptors and it is used in the treatment of insomnia.

Zolpidem belongs to a class of medications called sedative-hypnotics [3]. Literature survey reveals that four HPLC methods [4–7], one potentiometric method [8] one spectrophotometric method [9] have been developed for the estimation of zolpidem tartrate in human serum and tablet formulation. Stability testing plays an important role in the process of drug development. The purpose of stability testing is to provide confirmation on how quality of a drug substance varies with time under the influence of a variety of environmental factors such as temperature, humidity, and light and enables recommendation of storage conditions, and shelf life to be established. The assay of drug product in stability test sample needs to be determined using stability indicating method, as recommended by the international Conference on Harmonization (ICH).

The objective of the present work was to develop simple, rapid, accurate, specific and economic RP-HPLC stability indicating method [10] for the estimation of Zolpidem tartrate in bulk and tablet. The method was further validated as per ICH guidelines [11] for the parameters like precision, accuracy, sensitivity, and linearity. The results of analysis were validated statistically and by recovery studies. These methods of estimation of Zolpidem tartrate were found to be simple, precise, accurate and economic.

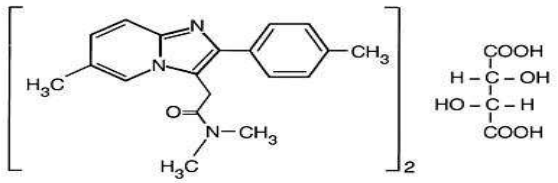

Figure no 01: Shows Chemical structure of Zolpidem tartrate

EXPERIMENTAL SECTION

Standard Drugs: Zolpidem tartrate

Chemicals and Reagents:

Zolpidem tartrate (Cadila Healthcare Limited, Goa), HPLC Water (Burgoyne Burbidge's and co.), Methanol for HPLC (Thermo Fischer Scientific India. Pvt. Ltd.), Acetonitrile for HPLC (Merck), Ammonium acetate (Acme Chemicals.) and Ammonia (Acme Chemicals).

Apparatus:

HPLC (Analytical technologies Ltd. UV detector), UV/VIS spectrophotometer (LABINDIA UV 3000+), pH meter (EQUIP-TRONICS, EQ-614A), Analytical balance (Sartorius, BSA2245-CW), SonicatorPipettes and Burettes (Borosil), Beakers Borosil.

Preparation of Standard Solution:

About 100 mg of Zolpidem tartrate is weighed and transferred to 100 ml volumetric flask, and was dissolved in mobile phase and the volume was made up to the mark with the same solvent. Further 10 ml of above solution was diluted to 100ml with mobile phase. Further 5ml is diluted to 50ml with mobile phase to get 10 µg/ml Zolpidem tartrate.

Preparation of Sample Solution:

A composite of 20 (Zolfresh) tablets was prepared by grinding them to a fine, uniform size powder. Weight equivalent to 100 mg of ZOL was accurately weighed and quantitatively transferred into a 100 ml volumetric flask, it was dissolved with mobile phase and the volume was made up to the mark with mobile phase. Further 10 ml of above solution was diluted to 100ml with mobile phase. Further 5ml is diluted to 50ml with mobile phase to get 10 µg/ml Zolpidem tartrate

Preparation of Phosphate buffer:

Accurately weighed 1.54g of Ammonium Acetate was taken in a 1000ml volumetric flask, dissolved and diluted to 1000ml with HPLC water and the volume was adjusted to pH 9 with ammonia.

Preparation of mobile phase:

Accurately measured 200 ml (20%) of above buffer, 400 ml (40%) of HPLC methanol , and 400 ml (40%) of HPLC Acetonitrile were mixed and degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation:

The Mobile phase was used as the diluent.

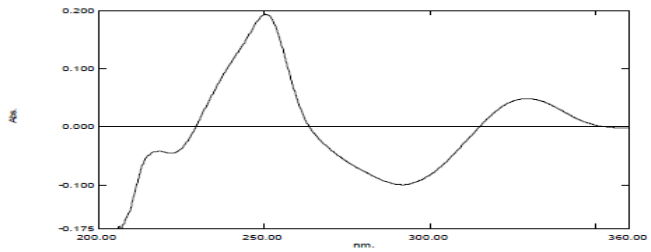

Fig. no.2: Shows UV spectrum of Zolpidem tartrate

From the above spectrum the wavelength selected for estimation of drug is 254 nm as λ max of Zolpidem tartrate.

Table no.01: Optimized Chromatographic Conditions:

Instrument used	Analytical technologies Ltd.
Temperature	Ambient
Column	Symmetry C8 (4.6 x 250mm, 5µm)
Buffer	1.54g of Ammonium Acetate in 1000ml HPLC water and pH adjusted with ammonia.
pH	9.0
Mobile phase	20% Ammonium Acetate buffer, 40% Methanol and 40% Acetonitrile
Flow rate	1 ml per min
Wavelength	254 nm
Injection volume	20 µl
Run time	10min.

Validation Parameter:
The following parameters were considered for the analytical method validation of Zolpidem tartrate in bulk form.

System Suitability:
Chromatograph the standard preparations (6 replicate injections) and peak area responses for the analyte peak was measured and the system suitability parameters are evaluated.

Accuracy:
For accuracy determination, three different concentrations were prepared separately i.e. 50%, 100% and 150% for the analyte and chromatograms are recorded for the same.

Precision:
The standard solution was injected for six times and the area was measured for all six injections in HPLC. The % RSD for the area of six replicate injections was found to be within the specified limits.

Robustness:
As part of the Robustness, deliberate change in the temperature and flow rate Variation was made to evaluate the impact on the method.

Linearity and range:
Linearity of the analytical method for assay by injecting the linearity solutions prepared in the range of 70 to 130% (7-13µg/ml) of test concentration, into the chromatograph, covering minimum 6 different concentrations.

Ruggedness:
Establish the ruggedness of the analytical method by using the assay of 6 different sample preparations of same batch by a different analyst using a different HPLC System.

RESULTS AND DISCUSSION

The developed method was validated based on ICH guidelines which detect and quantitate drug in bulk form with use of HPLC system equipped with UV detector.

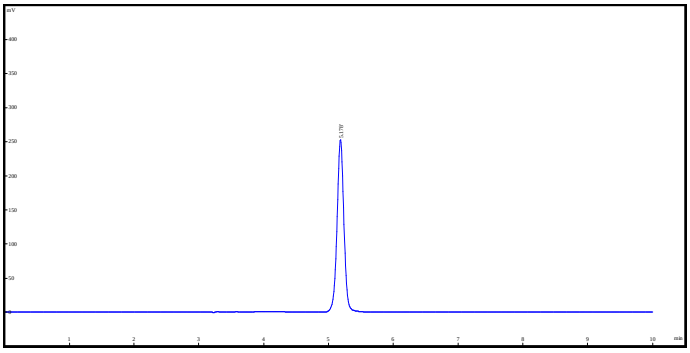

Figure no 03: Shows Chromatogram for Zolpidem tartrate

Table no 02: shows Method Development Parameters

PARAMETERS	ZOLPIDEM TARTRATE
Theoretical plates	11877
Tailing factor	1.00
Resolution factor	3.98
RSD of Area	0.007
RSD of Rt	0.01

Validation of developed RP-HPLC method:

Validation of an analytical method is the process to establish by laboratory studies that the performance characteristic of the method meets the requirements for the intended analytical application. Performance characteristics were expressed in terms of analytical parameters.

Accuracy:

The RP-HPLC method developed in the present study has been used to quantify Zolpidem tartrate. The average area was taken and % accuracy was calculated. The mean recoveries were found in the range of 99.97-100.02%. The results are presented in table no 03.

Table no 03: Shows Accuracy results for Zolpidem tartrate

Concentration of solution in %	Amount spiked (µg/ml)	Mean peak area of standard	Mean peak area of sample	Amount recovered	% Recovery
70%	7	1649770	1649409	9.99	99.97 %
100%	10	2325240	2325916	10.00	100.02 %
130%	13	3032223	3033054	10.00	100.02 %
Mean % Recovery					**100.00 %**

Precision:

The precision of the analytical method was studied by analysis of multiple sampling of homogeneous sample. The precision results were expressed as standard deviation or Relative standard Deviation

Table no 04: Shows Method Precision Results for Zolpidem tartrate

Drug	%RSD (Intraday)	%RSD (Interday)
Zolpidem tartrate	0.012	0.008

Linearity and Range:

The calibration curves were linear in the range 50-150 µg/ml. The correlation coefficient ('r') value was found to be >0.9997 for Zolpidem tartrate.

Table no 05: Shows Linearity results for Zolpidem tartrate

Drug	Concentration of solution in %	Amount in µg/ml	R^2
Zolpidem tartrate	70 to 130	7-13	0.9997

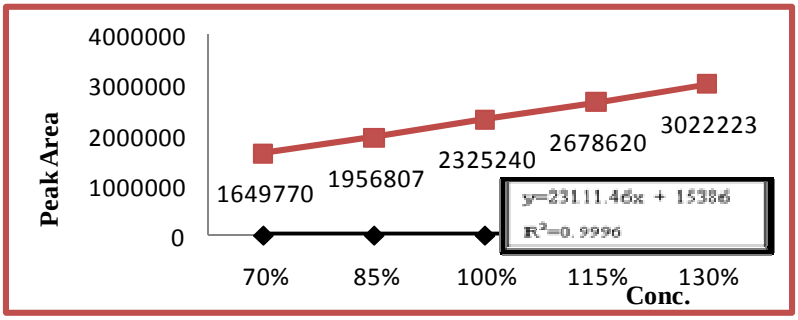

Figure no 04: Shows Calibration graph of Zolpidem tartrate

Robustness:

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

Table no 06: Shows Study of Robustness results for Zolpidem tartrate (Flow Rate)

Sr. No.	Flow rate	RT	Area	Content in mg
1	0.8	5.158	2359624	9.99
2	1.0	5.089	2325487	10.01
3	1.2	5.021	2317381	10.03

Table no 07: Shows Study of Robustness results for Zolpidem tartrate (Temperature)

Sr. No.	Temperature	RT	Area	Content in mg
1	20^0C	5.142	2318621	9.99
2	25^0C	5.027	2324718	10.02
3	30^0C	5.163	2327996	10.01

LOD:

The **limit of detection** is determined by the analysis of samples with known concentration of analyte and by establishing that minimum level at which the analyte can reliably detected, The LOD are calculated by formula LOD = 3.3 x SD/ b where, SD- standard deviation of the peak area of the drugs, b -is slope of the corresponding calibration curve. LOD for Zolpidem tartrate was 0.02µg/ml.

LOQ:

The **limit of quantification** is generally determined by the analysis of sample with known concentrations of analyte and by establishing the minimum level at which the analyte can be quantified with acceptable accuracy and precision, The LOQ are calculated by formula LOQ = 10 x SD/ b where, SD- standard deviation of the peak area of the drugs, b -is slope of the corresponding calibration curve. LOQ for Zolpidem tartrate was 0.05 µg/ml.

Table no 08: Shows LOD and LOQ results of Zolpidem tartrate

Parameters	Zolpidem tartrate
LOD	0.02 µg/ml
LOQ	0.05 µg/ml

Degradation Results:

Table no 09: Shows degradation studies of Zolpidem tartrate

S.No	Degradation	Rt	Area
1	Acid degradation	5.178	2361582
2	Base degradation	5.121	2359624
3	Thermal degradation	5.179	2344791
4	Photolytic degradation	5.181	2362103
5	Oxidative degradation	5.181	2361774

Table no 10: Shows validation summary of Zolpidem tartrate

S.NO	Parameter	Acceptance criteria	HPLC
1	Linearity range (µg/ml)	-	70-130(µg/ml)
2	Correlation coefficient	NLT 0.999	0.9997
3	No of Theoretical plates	NLT 2500	11877
4	Method precision	% RSD(NMT 2%)	0.012
5	Intermediate precision	% RSD(NMT 2%)	0.008
6	% recovery	98-102%	99.97-100.02%
7	LOD	-	0.02(µg/ml)
8	LOQ	-	0.05(µg/ml)

CONCLUSION

Method development and validation of Zolpidem tartrate was done by RP-HPLC method. The estimation was done by using Symmetry C18 (4.6 x 250 mm, 5µm, Make: Analytical Technologies). Mobile phase was used as Ammonium acetate Buffer, Methanol and Acetonitrile in (20:40:40) ratio at a flow rate 1 ml/min, retention time was 5.178 min. at λ max 254 nm. The linearity range of Zolpidem tartrate was found to be within 70-130 µg/ml. Mean recovery was 100.0 %, which is within 98-102%. Correlation coefficient value was 0.9997, % RSD was 0.057 % which is within the limit. These results show the method is accurate, precise, sensitive, economic and rugged. The HPLC method is more rapid. The proposed method can be successfully applied to estimate bulk drug and Tablet dosage form. The method was found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision.

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