AMERICAN JOURNAL OF PHARMTECH RESEARCH
Journal home page: http://www.ajptr.com/

Method Development and Validation of Zopiclone by RP-HPLC.

Rahul Khaire[1*], Mr. S. S. Dengale[1]
1.Pravara Rural College of Pharmacy, loni.

ABSTRACT

A simple, sensitive, rapid and selective isocratic reversed phase High Performance Liquid Chromatographic (HPLC) method has been developed for Zopiclone from bulk drug using a mobile phase consisting mixture of Acetonitrile : Buffer (pH 2.8) (80:20v/v) Composition of buffer: (0.272gm in 200ml HPLC water and pH adjusted to 2.8 using ortho Phosphoric acid) at the flow rate of 1.0 mL/min. A Cosmosil C_{18} (250 cm x 4.6 mm, 5 µm) column was used as stationary phase. The retention time of Zopiclone found to be 2.19 min. The eluent were detected at 303 nm. Linearity was observed in the concentration range of 50-250 ppm for Zopiclone. Percent recoveries obtained for Zopiclone were 98.66%. The correlation coefficient for Zopiclone was found to be 0.998. After performing analysis by different analysts, it was found that the RP-HPLC method for the determination of Zopiclone was found to be Rugged. Percent RSD for robustness was well within the acceptable USP limits, ensuring that the proposed method was robust. The LOD were 0.22 µg/ml Zopiclone,. For Zopiclone, the LOQ were found to be 0.86 µg/ml. This demonstrated that the developed RP-HPLC method was simple, linear, precise, accurate, robust, and Rugged, could be conveniently adopted for the routine quality control analysis of Zopiclone.

Keywords: Zopiclone, HPLC Method, Mobile phase.

*Corresponding Author Email: rahuldkhaire@gmail.com
Received 15 August 2015, Accepted 01 June 2016

Please cite this article as: Khaire R*et al*., Method Development and Validation of Zopiclone by RP-HPLC. American Journal of PharmTech Research 2016.

INTRODUCTION

High performance liquid chromatography:

HPLC it is a separation technique where solutes moves within a column containing a micro particulate as a stationary phase at rates dependent on their distribution ratios.

Modes Of Separation In HPLC

Different modes of separation in HPLC. They are as follows

Normal Phase Chromatography (NPC)

It is also known as Adsorption Chromatography. In the Normal Phase Chromatography, the stationary phase is polar (silica or alumina) and the mobile phase is non-polar in nature. In this technique, nonpolar compounds travel faster and are eluted first. This is because of the lower affinity between the non-polar compounds and the stationary phase. Polar compounds are retained for longer times because of their higher affinity with the stationary phase.

Reversed Phase Chromatography (RPC)

In this technique, the stationary phase is non-polar hydrophobic packing with octyl or octadecyl functional group bonded to silica gel and the mobile phase is polar solvent. [1,2,3]

MATERIALS AND METHOD

Zopiclone:

Systematic (IUPAC) name

(RS)-6-(5-chloropyridin-2-yl)-7-oxo-6,7-dihydro-5H-pyrrolo[3,4-b]pyrazin-5-yl 4-methylpiperazine-1-carboxylate .

Category

Central nervous system depressant and non benzodiazepine sedative and hypnotic.

Molecular Structure

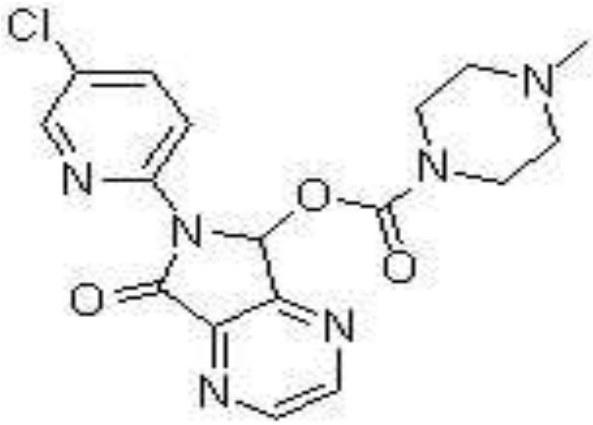

Figure 1: Structure of Zopiclone

Appearance

Fine white or slightly cream crystalline powder.

Table 1:Drug sample suppliers & Manufacturer

Sr. No.	Name of Drugs	Drug supplies & Manufacturer.
1	Zopiclone	Glenmark Pharmaceuticals (Mumbai), Maharashtra, India.

METHOD

Selection of analytical wavelength:

Standard stock solution of Zopiclone:

10mg of Zopiclone were accurately weighed, transferred to separate 10 ml volumetric flasks, dissolved in the mobile phase and dilute to volume with the same solvent mixture to furnish stock solutions containing 1000µg/ml of Zopiclone. 1 ml of above solution transferred in 10 ml volumetric flask and the volume was made with diluents. The concentration of Zopiclone is 100µg/ml.[4]

Determination of λmax of Zopiclone:

Standard stock solution of Zopiclone was diluted separately with diluents to obtain final concentration of 10 µg/ml. solution was scanned using UV-Visible Spectrophotometer in the spectrum mode between the wavelength range of 400 nm to 200 nm.

Determination of Absorption maxima:

By appropriate dilution of standard drug solutions with acetonitrile, solutions containing 10 µg/ml Zopiclone were scanned separately in the range of 200-400 nm to determine the wavelength of maximum absorption for the drugs.

HPLC method development:

Selection of Mobile phase:

Zopiclone was injected into the HPLC system and run in different solvent systems. Mixture of different solvents were tried in order to determine optimum chromatographic conditions for effective separation. After several permutation and combination, it was found that mixture of Acetonitrile : Buffer, with ortho phosphoric acid to adjust the p^H,gives satisfactory results as compared to other mobile phases. Finally, the optimal composition of the mobile phase contains about 80 volume of Acetonitrile and 20 volume of Buffer [pH 2.8], as it gave high resolution of Zopiclone with minimal tailing.

Preparation of mobile phase:

Preparation of Buffer:

An accurately weighed quantity of about 0.272gm of Potassium Di hydrogen Phosphate was taken in 500 ml volumetric flask dissolved in sufficient quantity of HPLC water, then sonicated for 15 min and diluted to 200ml with the HPLC water. Then adjust the p^H up to 2.8 with orthophosphoric acid and filter through a 0.45µm membrane filter, gives the formation of buffer.

Mobile phase

Finally, the optimal composition of the mobile phase contains about 20 volume of buffer and 80 volume of Acetonitrile. [Acetonitrile : Buffer(80:20)]

Preparation of standard stock solution:

10mg of Zopiclone were accurately weighed, transferred to separate 10 ml volumetric flasks, dissolved in the mobile phase and dilute to volume with the same solvent mixture to furnish stock solutions containing 1000µg/ml of Zopiclone. 1 ml of above solution transferred in 10 ml volumetric flask and the volume was made with diluents. The concentration of Zopiclone is 100µg/ml.[5]

Loading of mobile phase:

Filtered & degassed mobile phase was loaded in the reservoir. Priming was done for each freshly prepared mobile phase.

Baseline stabilization:

The detector was turned on for an hour before the actual run in order to obtain the stable UV light. The mobile phase run was started at desired flow rate & the run was continued until the stable baseline was obtained.

Loading of samples:

Well prepared & filtered samples of Zopiclone were loaded into the Rheodyne injector port using a syringe & the sample was then injected.

RESULTS AND DISCUSSION

UV analysis for detection of wavelength:

Determination of λmax of Zopiclone:

The standard solution of Zopiclone was scanned at different Conc. in the range of 200-400nm and the λ max was found to 303nm.[6]

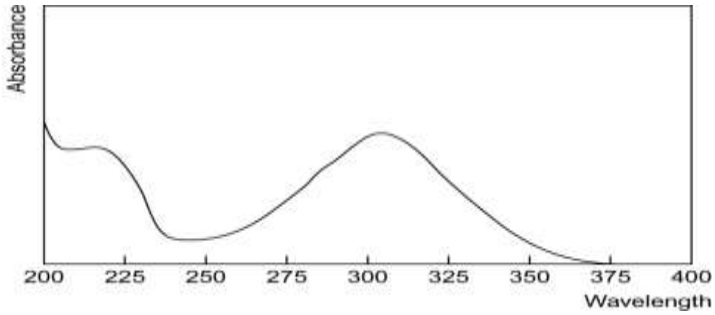

Figure: 1 UV spectrum for Zopiclone

HPLC METHOD DEVELOPMENT:

Table 2: optimized chromatographic Condition for RP-HPLC method.

Parameters	Values
Column	Cosmosil C_{18}
Wavelength	303nm
Flow rate	1.0ml/min
Injection volume	20µl
Temperature	Ambient
Run time	10 min

Assay of Zopiclone:

Table 3 : Chromatograph for standard drug of Zopiclone.

Rank	Time	Conc	Area	Resolution	T.PlateNum	Asymmetry
1	2.19	100.0000	138158.26	0.00	2351	0.81

Table 4: Chromatograph for Tablet sample solution of Zopiclone

Rank	Time	Conc	Area	Resolution	T.PlateNum	Asymmetry
1	2.17	100.0000	132638.57	0.00	2366	0.79

Table 5: Data for Assay of Zopiclone

Wt. of Std (mg)	**Area of standard**	**Area of sample**	**Purity of the Std (%)**
10	138158.26	139472.83	100%
	135516.00	138068.17	
	137437.66	135531.26	
	132638.57	135935.03	
	134903.14	138679.03	
	137279.31	136615.43	
Mean	134967.24	136548.90	
SD	2274.04	1577.88	
RSD	0.44	0.30	

Percentage Assay obtained for Zopiclone is 100 % (Standard-NLT 98.0 and NMT 102.0%). As the result obtained is within the limits, hence this assay method used to perform the validation.

Table 6: System Suitability Test:

Sr. No.	**Area of standard**	**Retention time (R_t)**	**USP tailing (T_f)**	**Theoretical plate count (N)**	**Resolution (Rs)**
1	132028.80	2.19	1.07	2163	0.00
2	135516.00	2.19	1.03	2351	0.00
3	137437.66	2.18	1.09	2254	0.00
4	132638.57	2.18	1.06	2284	0.00
5	134903.14	2.18	1.07	2415	0.00
Average	134504.83	2.18	1.06	2293.4	0.00
SD	2198.05				
% RSD	0.4443				

Acceptance Criteria:

RSD should not be more than 2.0 % for five replicate injections of standard

USP Tailing Factor is not more than 2.0.

The column efficiency as determined as number of theoretical plates should be more than 4500.

Conclusion:

%RSD of the was found to be: Zopiclone: 0.4443

Number of Theoretical plates was found to be : Zopiclone: 2293.4

Tailing factor found was to be: Zopiclone: 1.06

Table 7: Analysis Data for Tablet Formulation

Drug	Label claim (mg/tab)	Amount found (mg/tab)	Percent label claim	% RSD
Zopiclone	7.5	7.4	98.66	1.045

Analysis of Tablet formulation of Zopiclone: After analysis of Zopiclone tablet it was found that the amount of Zopiclone found after calculation, was within the limit of label claim as mentioned in table.

VALIDATION OF THE DEVELOPED RP-HPLC METHOD- [7,8]

Table 8: Specificity:

Zopiclone	Area	Amount added(mg)	Amount recovered (mg)	Percent recovery (%)	SD	RSD
Drug Sample	139090.46	5	4.8	96	0.7020	0.12
	138068.17	7.5	7.3	97.33		
Tablet sample	138074.11	5	4.7	94	0.6989	0.11
	136335.37	7.5	7.3	97.33		

Results and discussions:

No interference from any of the excipients was found at retention times of the examined drugs. In addition, the chromatogram of each drug in the sample solution was found identical to the chromatogram received by the standard solution at the wavelengths applied. These resultsdemonstrate the absence of interference from other materials in the pharmaceutical formulations and therefore Confirm the specificity of the proposed method.

Precision

Table 9: Intra-Day variability for Zopiclone

Table: Intra-Day variability for Zopicloneclone	Trial No	Area	Amount added (mg)	Amount recovered (mg)	Percent recovery (%)	SD	RSD
Drug Sample	1	134464.57		9.6	96	0.3572	0.3723
	2	138068.17	10	9.6	96		
	3	135531.26		9.8	98		
Tablet Sample	1	134464.57		9.8	98	0.3582	0.3633
	2	138068.17	10	9.6	96		
	3	135531.26		9.7	97		

Table 10: Inter-Day variability for Zopiclone

Zopiclone	Trial No	Area	Amount added(mg)	Amount recovered(mg)	Percent recovery(%)	SD	RSD
Drug	1	135935.03		9.5	95		
Sample	2	138679.03	10	9.6	96	0.2721	0.2875
	3	136615.43		9.6	96		
Tablet	1	135935.03		9.8	98		
Sample	2	138679.03	10	9.7	97	0.2762	0.2865
	3	136615.43		9.3	93		

Results and discussions:

The RSDs for intra-day and inter-day precision were not more than 2.0% for Zopiclone. The low RSD values indicate the repeatability and reproducibility of the method. Therefore, as per the ICH guidelines, this HPLC method for the determination Zopiclone was precise.

Linearity:

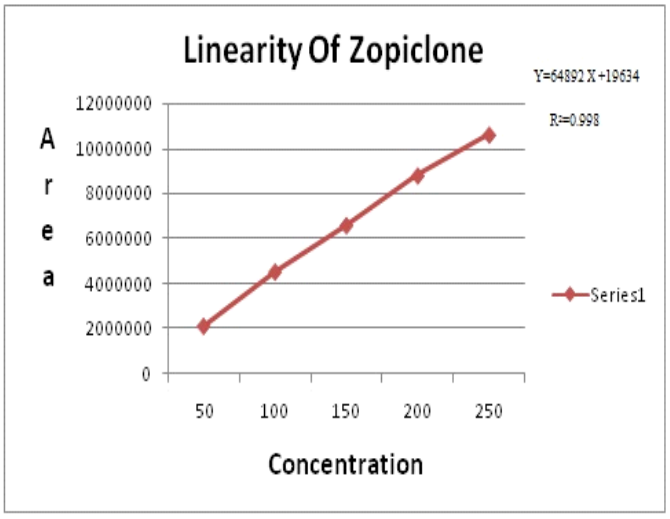

Figure 2: Linearity graph for Drug Sample Zopiclone

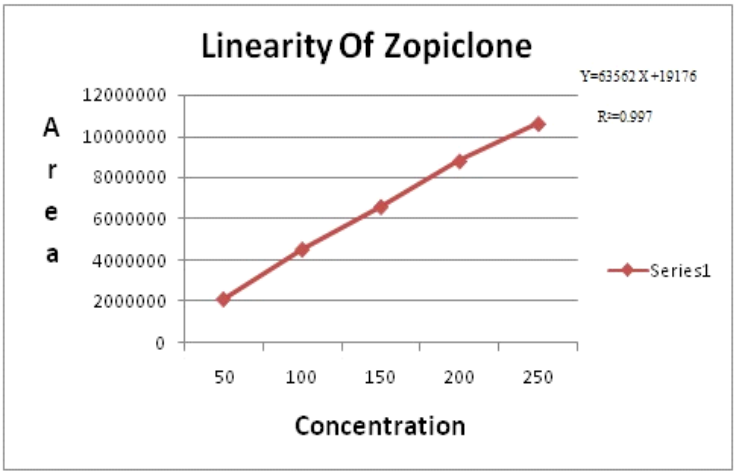

Figure 3: Linearity graph for Tablet Sample Zopiclone

Results and Discussions

The calibration curves exhibited linear relationship of peak area to Concentration in the range 50-250 µg/ml for Zopiclone. The regression coefficients (r^2) for Zopiclone were 0.998, maintaining good correlation close to unity. The graph of Concentration Vs Average area was plotted which is showing straight line passing through all points. So as per ICH guidelines, the proposed HPLC method for the determination of Zopiclone was found to be linear.

Table 11: Accuracy (Recovery):

Zopiclone	Accuracy Levels	Area	Amount added (mg)	Amount recovered (mg)	Percent recovery (%)	SD	% RSD
Drug	50ppm	140177.03	0.05	0.05	100	0.967	0.98
Sample	150ppm	139040.66	0.15	0.14	93.33		
	250ppm	135786.65	0.25	0.24	96		
Tablet	50ppm	13777.03	0.05	0.04	99	0.987	0.96
Sample	150ppm	139090.46	0.15	0.13	86.66		
	150ppm	135278.11	0.25	0.23	92		

Results and Discussions: Accuracy was checked with standard drugs by placebo spiking method at three different Concentration levels (multi-level recovery). Recovery of standard drugs added was found to be 96.44% for , indicating that the proposed method is accurate for the simultaneous estimation of Zopiclone drug products in presence of their degradation products and excipients.

Table 12: Ruggedness (At Flow Rate 1.1ml/min)

Zopiclone	Area	Amount added(mg)	Amount recovered(mg)	Percent recovery(%)	SD	% RSD
Drug Sample	142638.57	10	9.5	95	0.3110	0.34
	134993.14	10	9.6	96		
	137229.31	10	9.6	96		
	139391.10	10	9.7	97		
Tablet	139956.54	10	9.3	93	0.2938	0.29
Sample	132999.22	10	9.5	95		
	136977.42	10	9.6	96		
	131927.60	10	9.8	98		

Results and discussions:

To evaluate the ruggedness of the proposed RP-HPLC method, the analysis was performed by different analysts and employing different brands of chemicals and solvents. Overall RSD for results obtained from different analysts are within limits. Therefore, the HPLC method for the determination of Zopiclone was found to be Rugged.

Limit of Detection and Quantitation:

The limits of detection (LOD) and quantification (LOQ) were determined separately, on the basis of the standard deviation of the y intercept and slope of the calibration plots. The LOD were 0.22 µg/mL for Zopiclone. For Zopiclone, the LOQ were found to be 0.86. At these levels, RSD values were less than 2%, in accordance with ICH guidelines.

CONCLUSION

Based on the results obtained, it can be concluded that the proposed RP-HPLC method for the determination of Zopiclone in bulk drug & solid dosage form is simple, linear, precise, accurate, robust, and rugged. The utility of the developed methods have been demonstrated by analysis of bulk drug & solid dosage form. Hence, the proposed method can be used for quantitative determination of these ingredients in bulk drug & dosage form.

ACKNOWLEDGEMENT

Successful completion of a project is not achieved single handedly; it is always backed by the constant silent support and guidance of several well wishers and loved ones. First and foremost, I bow before the almighty god for showering his blessings on me and giving me the strength to carry out the present work with utmost dedication and enthusiasm.

With great pleasure and deep sense of gratitude, I express my most pleasant and modest thank to my respected guide Mr. S. S. Dengale Asst. Prof. & Head Dept. of Pharmaceutical Chemistry, Pravara Rural College of Pharmacy, Loni. For his valuable support, inspiration and encouragement through my project work. His simplicity, untiring and meticulous guidance and provision of hardwork environment will be cherished in all walks of my life. It was an enriching experience to work under him.

REFERENCE

1. Sethi P.D. HPLC ‘High Performance Liquid Chromatography’, Quantitative Analysis of Pharmaceutical Formulations, 1st Ed., CBS Publishers and Distributors, New Delhi, 2001,Page No. 3-39, 116-120.
2. M.Z. Ahmad and M. Ali. Textbook of Pharmaceutical Drug Analysis. 1stEdn. 2009, pp.254-264.
3. Skoog D. A., Holler F. J., Nieman T. A., Principles of Instrumental Analysis, 5th Edition, Eastern Press, Bangalore, 2004, Page No. 1-2, 674-688,695-698.
4. Sharma B.K, Instrumental Methods of Chemical Analysis, Introduction to analytical chemistry 21st ed., Goel Publishing House, Meerut, 2002, Page No. 3-5, 10.

5. Willard, H. H., Merritt, L. L. Jr., Dean, J. a. Frank, A. S., Instrumental methods ofanalysis, CBS Publishers and distributors, New Delhi, 7th edition, 1986, Page No. 1- 53, 513-522, 530-535.
6. Meng M., Rohde L., Capka V. and Bennett P., Development and Validation for the Quantitation of Eszopiclone in Human Plasma Using LC/MS/MS. Tandem Labs., Chiral Chromatographic Method, 2007, 1-14.
7. Determination of Zopiclone in tablets by HPLC and UV-spectrophotometry; Journal of Pharmaceutical and Biomedical Analysis; 2000; 23(5): 819-823.
8. RP-HPLC Method for the Estimation of Zopiclone in Tablet Dosage Form; International Journal of Pharmaceutical Research; 2011; 3(1): 49-51.