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AVALIDATEDSTABILITY-INDICATINGHPLCASSAYMETHODFOR ZOPICLONE IN BULK DRUG

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ABSTRACT

An isocratic reversed phase stability-indicating high performance liquid chromatographic (HPLC) assay method was developed and validated for quantitative determination of Zopiclone in bulk drugs. Zopiclone is a non-benzodiazepine compound and its mechanism of action based on modulating benzodiazepine receptors. It is hypnotic agent and used in treatment of insomnia. It is classified in category of Z-drugs. Method developed on column Cosmosil C18, 250 x 4.6 mm, 5 µm or equivalent, and the mobile phase containing 0.5% glacial acetic acid. The developed method was validated with respect to linearity, accuracy, precision, system suitability, selectivity, robustness prove the stability indicating ability of the method.

KeyWords: HPLC,Zopiclone,non-benzodiazepine,chromatographic techniques.

1. INTRODUCTION

Zopiclone is chemically non-benzodiazepine compound [1]. It is hypnotic in nature, which is found effective and been used for treatment in insomnia [2]. Zopiclone suppresses the brain activity and helps patient to sleep. It is classified in Z-drugs like that of benzodiazepines core structure containing drugs and barbiturates but chemically unrelated with them as shown in figure 1. Zopiclone during its mechanical action interacts with gamma-amino butyric-benzodiazepine receptor complex. Zopiclone acts on benzodiazepine binding site particularly on α1, α2, α3 and α5 GABAA and exhibits negative action of GABA [3-5]. Molecular formula of Zopiclone is C17H17ClN6O3. Structural formula is depicted in figure 1 and molecular weight is 388.8g/mol.

To analyze any drug most desirable method must be easy and sensitive with cost-effective. .In the present work we developed and validated a simple and sensitive high performance liquid chromatographic method for the determination of Zopiclone as bulk or as tablet dosage [6], according to the ICH guidelines [7-9].

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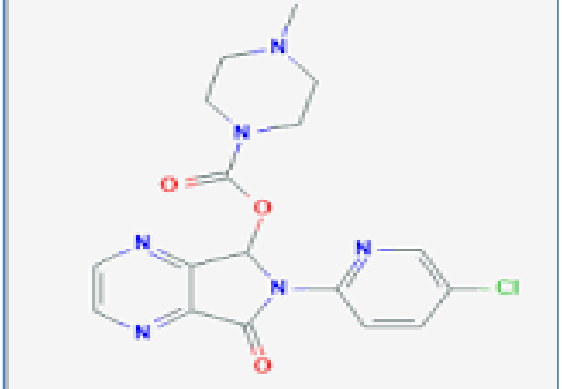

Fig.1.Chemical Structure of Zopiclone.

2. LITERATURE SURVEY

Literature survey revealed that many HPLC methods have been reported for estimation of Zopiclone in bulk as well as pharmaceutical preparations [10-16].

3. MATERIALS AND METHODS

The separation of the analyte was done on HPLC system with, column Cosmosil C18, and 250 x 4.6mm 5µm or equivalent column. The instrument was equipped with a pump (2695), injector, PDA Detector (2996) and column oven. Data gaining was done by using Empower software. Degassing of the mobile phase was done by using an ultrasonic bath sonicator whenever necessary. For weighing the materials a Mettler Toledo (XS 205 dual range) electronic balance was used. Class 'A' Borosil glassware were employed for volumetric and general purpose in the study. The reference sample of Zopiclone was obtained from Lupin Pvt. Ltd. Aurangabad. The tablets of Zopiclone were obtained from the local market. .Sodium lauryl sulphate (AR grade, Merck), Sodium hydrogen phosphate (Merck), Acetonitrile (HPLC grade, Sigma Aldrich), ortho phosphoric acid (Merck), water (Milli-Q / HPLC grade) were used. Chromatographic conditions were used are shown in **table 1.**

4. CHROMATOGRAPHIC CONDITIONS AND PREPARATION OF SOLUTIONS:

Preparation of buffer solution

Weighed accurately about 8.1 gm/litre of sodium lauryl sulphate and 1.6 gm/litre of sodium dihydrogen phosphate and diluted upto the mark with water.Adjusted the pH 4.0 with10%v/v solution of ortho phosphoric acid.

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Preparation of mobile phase:

Buffer	:	Acetonitrile (separate lines)
62	:	38

Preparation of Zopiclone working standard solution:

Weighed accurately 100mg of Zopiclone working standard and transfer into a 100ml volumetric flask, dissolved and diluted up to the mark with diluents.

Preparation of Zopiclone sample solution:

Weighed accurately 100mg of Zopiclone sample and transfer into a 100ml volumetric flask, dissolved and diluted up to the mark with diluents.

Preparation of standard solution:

Weighed accurately 50.58 mg of standard and transferred it into a 100 ml volumetric flask, dissolved and diluted the volume up to the mark with diluents. Injected six replicate injections were of Zopiclone working standard solution.

Preparation of sample solution:

Weighed accurately 99.51 mg of test sample and transferred it into a 100 ml volumetric flask, dissolved and diluted the volume up to the mark with diluents. Prepared six samples solutions separately in similar manner. Calculated the Assay of each sample of Zopiclone by comparing against the working standard. Calculated % RSD of assay values.

$$\text{Assay} = \frac{At}{As} \times \frac{Ws}{Wt} \times \frac{P}{(100 - LOD)} \times 100$$

Where,

At=Areaofprincipalpeakinsamplesolution,

As=Averageareaofprincipalpeakinstandardsolution,

Ws = Weight of working standard in mg,

Wt=Weightof sampleinmg,

P=%Potencyofworkingstandardonasis basis.

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Table1.Optimized Chromatographic conditions.

1.	Instrument	AgilentHPLCSeries1200withUV detectorandautosampler
2.	Column	CosmosilC18250x4.6mm5µmor equivalent.
3.	Mobile phase	Buffer:Acetonitrile(62:38)
4.	Flowrate	1.5ml/ min.
5.	Wavelength	303 nm
6.	Injectionvolume	20µl
7.	Column temperature	30°C
8.	Injectortemperature	5°C
9.	Diluent	Mobile phase
10.	Run Time	60 minutes

5. METHOD VALIDATION

The method was validated as per ICH guidelines.The parameters determined for validation were specificity, precision, accuracy, robustness, linearity, Limit of Quantification and Limit of Detection, system suitability and stability of analytical solution.

SPECIFICITY

The method specificity was assessed by comparing the chromatograms obtained from a saline solution containing a mixture of most commonly used excipients without the drug and another solution containing the excipients with the drug. These solutions were prepared in the mention diluents. The mixtures were filtered before injection. The saline solution and the sample solution (blank and the drug) were injected into HPLC system and the relevant chromatograms observed.

METHOD PRECISION

System precision:

The system precision of the method was determined by injecting six replicates of standard solution of Zopiclone into HPLC system.

Method precision:

The precision of the procedure was determined by repeatability. In this six sample preparations

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were made from a single batch of Zopiclone tablets and analyzed as per the proposed method.

Intermediate precision (Ruggedness):

Ruggedness of the method was performed by analyzing six sample preparations of same batch used under method precision as per proposed method by different analysts using different instrument and on different day. The amount of Zopiclone in Zopiclone tablets was determined and % RSD for % assay of was calculated, for six preparations.

Accuracy:

The accuracy was verified with known amount of Zopiclone (API) between 98.5% and 100% of test concentration prepared in triplicate at each level.Amount of Zopiclone was quantified and % recovery was calculated from amount found and actual amount added.% Recovery at each level was calculated.

Linearity:

Linearity of method was performed using the standard solution in a range of 80ppm to 240ppm [50% - 150% of the test concentration].

Stability in analytical solution:

Stability of Zopiclone in analytical solution was determined by analyzing sample solution initially and also at different time intervals up to 27 hrs when the sample was stored at room temperature.

Robustness:

To evaluate robustness of the method following variations were made and the samples were analyzed in triplicate. Change in Flow rate by (10%), change in Organic content variation in mobile phase (± 2 mM). System suitability was evaluated in each condition and results were compared with method precision results.

Limit of Detection and Limit of Quantification: Limit of detection (LOD) is the lowest concentration of analyte that gives a measurable response. LOD is determined based on signal to noise ratio(S/N) of three times typically for HPLC methods.The limit of quantification (LOQ) is the lowest concentration that can be quantified reliably with a specified level of accuracy and precision.

6. RESULTSANDDISCUSSIONS:

Mixture of Buffer: acetonitrile (62:38) at a flow rate1.5ml/minute were found as suitable solvent system.

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Table2.Observation table for Method Precision data (sample).

Sample No.	*Wt. of sample (mg)*	*Retention Time*	*Area*	*Assay(%) (as such basis)*	*Assay (%) (on dried basis)*
1	100.55	27.01	5125.25	99.72	99.86
2	100.85	27.02	5143.10	99.77	99.91
3	100.75	27.01	5138.74	99.78	99.92
4	100.86	27.01	5144.45	99.79	99.93
5	100.88	27.02	5148.74	99.85	99.99
6	100.92	27.01	5150.15	99.84	99.98
AVG	--	27.01		99.79	99.93
%RSD	--			0.05	0.05

Result:

% RSD of assay values of Zopiclone is 0.05 %.

Acceptance criteria

% RSD of assay values should not be more than 2.0 %.

ACCURACY

Accuracy of Zopiclone was studied by sample solutions prepared at three different levels as given below.

Preparation of Zopiclone standard

Weighed 99.15 mg of Zopiclone standard and transferred intoa100 ml volumetric flask. Dissolved and diluted up to the mark with diluents.

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Table3.Observation: Accuracy data-Standard

Working Standard	*Wt. of Standard (mg)*	*Area*
Injection-1	99.15mg	5054.20
Injection-2	--	5052.99
Injection-3	--	5056.03
Injection-4	--	5055.18
Injection-5	--	5057.31
Injection-6	--	5064.94
AVG	--	5056.78
%RSD	--	0.08

Table3.1AccuracyLevel-1

Accuracy Level	*Wt.ofsample (mg)*	*Area*	*Assay (assuch basis)*	*Assay (on dried basis)*
Level-1	71.51	3638.80	99.62	99.76
	71.43	3635.99	99.66	99.80
	71.49	3637.88	99.63	99.77
	AVG	--	--	
	%RSD	--	--	

Table3.2AccuracyLevel-2

Accuracy Level	*Wt.ofsample (mg)*	*Area*	*Assay (assuch basis)*	*Assay (on dried basis)*
Level-2	102.75	5234.95	99.75	99.89
	102.65	5228.03	99.71	99.85
	102.66	5232.55	99.79	99.93
	AVG	--	--	
	%RSD	--	--	

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Table3.3AccuracyLevel-3

Accuracy Level	Wt.ofsample (mg)	Area	Assay (assuchbasis)	Assay (ondriedbasis)
Level-3	129.55	6573.14	99.34	99.47
	130.55	6600.60	98.99	99.12
	129.35	6565.55	99.37	99.51
	AVG	--	--	
	%RSD	--	--	

Result:

% RSD of peak area of Zopiclone working standard is 0.08%.

Accuracy of each sample is between 98.5 to 100.0%.

Acceptance criteria:

% RSD of peak area of Zopiclone should not be more than 2.0%.

Accuracy of assay should be between 98.5-100.5 %.

Linearity:

Linearity of Zopiclone was studied by injecting solutions prepared at five different levels from working standard stock solution as given below.

Preparation of Zopiclone working standard stock solution (1000 ppm):

Weighed accurately 100 mg of Zopiclone working standard and transferred to 100ml volumetric flask. Dissolved and diluted up to the mark with diluents, mixed well (80 ppm)

LinearityLevel-1:

Transferred 2.0 ml of Zopiclone working standard stock solution (1000 ppm) into 25 ml volumetric flask and diluted up to the mark with diluents (80 ppm).

LinearityLevel-2:

Transferred 3.0 ml of Zopiclone working standard stock solution (1000 ppm) into 25 ml volumetric flask and diluted up to the mark with diluents (120 ppm).

LinearityLevel-3:

Transferred 4.0 ml of Zopiclone working standard stock solution (1000 ppm) into 25 ml volumetric flask and diluted up to the mark with diluents (160 ppm).

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LinearityLevel-4:

Transferred 5.0 ml of Zopiclone working standard stock solution (1000 ppm) into 25 ml volumetric flask and diluted up to the mark with diluents (200 ppm).

LinearityLevel-5:

Transferred 6.0 ml of Zopiclone working standard stock solution (1000 ppm) into 25 ml volumetric flask and diluted up to the mark with diluents (240 ppm).

Injected these five levels in three replicates. Calculated % RSD and average peak area of theselevels.

Table4.Observation:Linearity data.

InjectionNo.	***Level-1 (80ppm)***	***Level-2 (120ppm)***	***Level-3 (160ppm)***	***Level-4 (200ppm)***	***Level-5 (240ppm)***
1	2566.18	3856.52	5101.55	6447.51	7598.19
2	2572.08	3816.34	5103.87	6439.48	7608.23
3	2575.46	3818.24	5111.38	6436.51	7600.96
AVG	2571.24	3830.37	5105.60	6441.17	7602.46
%RSD	0.18	0.59	0.10	0.09	0.07
--	--	--	--	Correlation Coefficient(r)	1.000

Result:

Correlation of Coefficient (r) of Zopiclone is1.000

Acceptance criteria:

Correlation of Coefficient (r) for Zopiclone should not be less than 1.000

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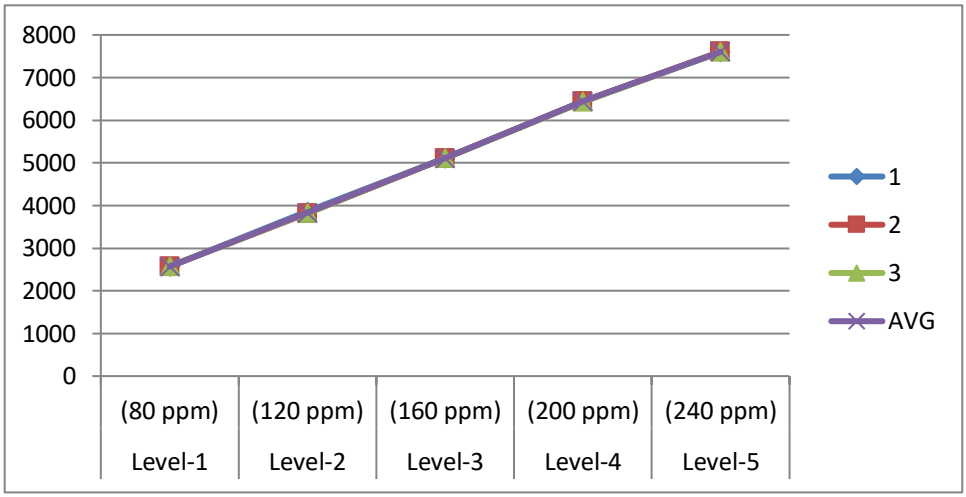
Fig.2.Linearity graph of Zopiclone.



Limit of Detection and Quantification

The limit of detection and the limit of quantification was estimated by injecting serial dilutions of less than1.0 ppm of Zopiclone six replicates and calculated % RSD of Zopiclone.

Observations:LOD/LOQ:Zopiclone

Sample	***Level – 1***	***Level – 2***	***Level – 3***	***Level – 4***	***Level – 5***
ppm	10 ppm	5 ppm	3 ppm	2 ppm	1 ppm
	Area	Area	Area	Area	Area
Injection - 1	4.35	3.63	2.10	1.31	Not detected
Injection - 2	4.31	3.61	2.12	1.24	Not detected
Injection - 3	4.34	3.45	2.19	1.22	Not detected
Injection - 4	4.41	3.59	2.17	1.16	Not detected
Injection - 5	4.38	3.66	2.18	1.33	Not detected
Injection - 6	4.39	3.73	2.15	1.21	Not detected
Average	4.3	3.61	2.15	1.24	--
%RSD	0.68	0.19	0.04	2.1	--

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Results:

Limit of detection for Zopiclone is 0.05 ppm and limit of quantification is 0.10 ppm.

Acceptance Criteria:

LOD would be the lowest concentration of analyte which can be detected but when % RSD of six replicate injections should be more than 10%. LOQ would be the lowest concentration of analyte which can be quantified but when % RSD of six replicate injections should be less than 10%.

Fig.3 Chromatogram of Zopiclone Standard

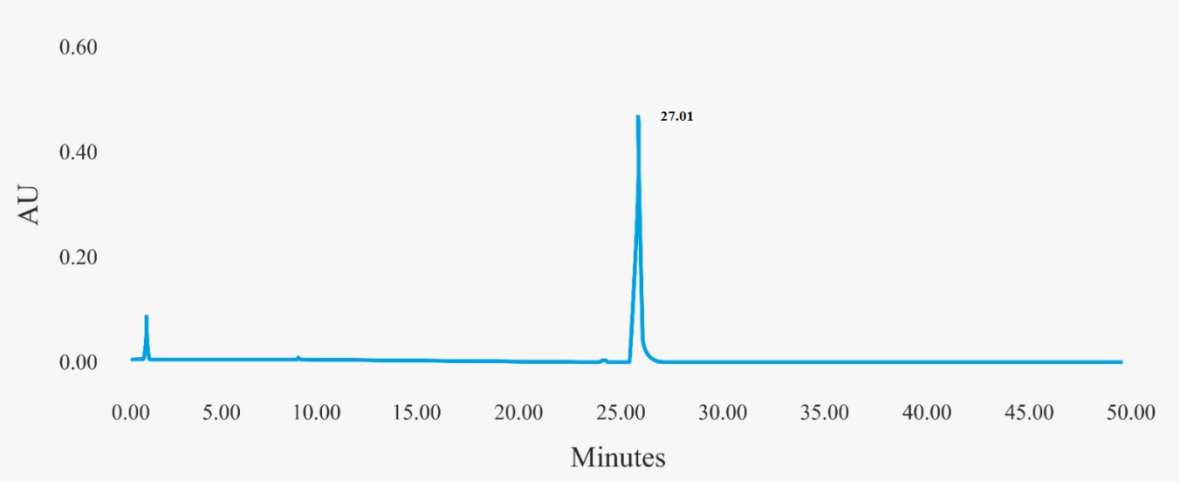
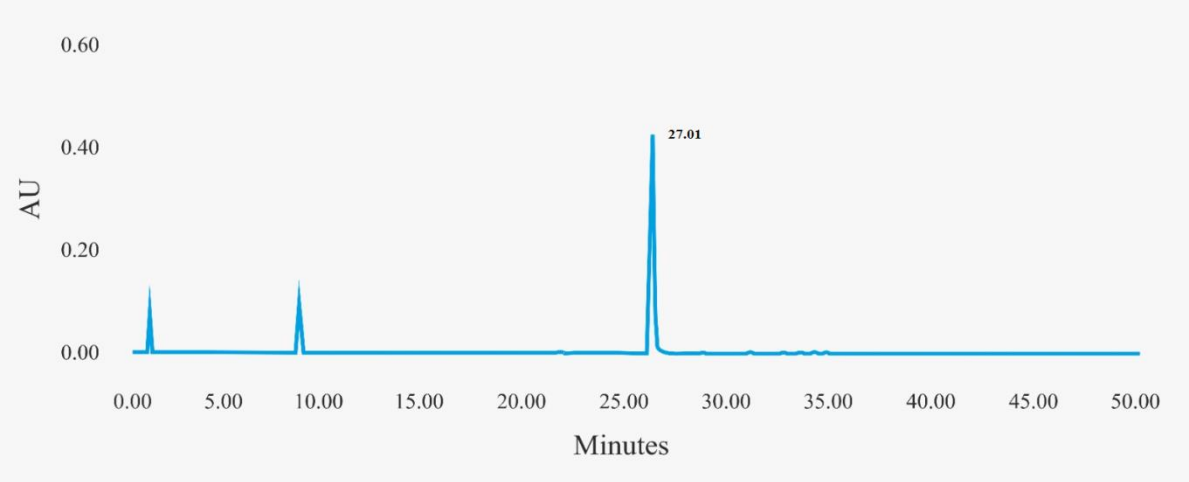


Fig.4 Chromatogram of Zopiclone Sample



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7. CONCLUSION

The method developed for quantitative determination of Zopiclone is rapid, precise, accurate, economic and selective. The method was completely validated showing satisfactory data for all method-validated parameters tested. The developed method can be conveniently used for the assay determination of Zopiclone in bulk drugs and pharmaceutical dosage form.

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