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HPTLC Method Development and Validation of Zolpidem Tartrate in Bulk and Marketed Formulation

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ABSTRACT

High performance thin layer chromatography (HPTLC) offers many advantages over HPLC. It reduces the cost of analysis as compare to HPLC. The mobile phase consumption per sample is extremely low in HPTLC, hence reducing the acquisition and disposal cost. Considering the cost and suitability of analysis for estimation of zolpidem tartrate in bulk and its marketed formulation, HPTLC method was developed and validated. The Camag HPTLC system, employed with software winCATS (ver.1.4.1.8) was used for the proposed analytical work. Planar chromatographic development was carried out with the help of Silica Gel 60 F_{254} precoated TLC plates. Sample application was facilitated by Linomat 5 applicator. After sample application plates were subjected for ascending development in twin trough chamber of 10×10 dimension, using 10 ml of solvent system. The optimised mobile phase was composed of ethyl acetate: methanol: acetonitrile (7:1.5:1.5 v/v/v). In post- development, the plates were air dried and then scanned densitometrically using a UV detector at 298 nm in absorbance mode. In HPTLC densitogram well defined peak was obtained for zolpidem tartrate with peak start position at 0.55 R_f , max position at 0.59 R_f and end position at 0.63 R_f. The optimal R_f value for zolpidem tartrate was found to be 0.58. Performance characteristics of HPTLC method for estimation of zolpidem tartrate in bulk and its marketed dosage form were statistically validated as per the recommendations of ICH guidelines of analytical method validation. The HPTLC method was found to be linear across the range 200- 800 ng/spot. The LOD and LOQ values were found to be 16.99 and 51.50 ng/spot respectively. The method was found to be accurate, precise, robust and economical for the analysis of zolpidem tartrate from bulk and its formulation.

Keywords: HPTLC, zolpidem tartrate, marketed dosage form, ICH, analytical method validation.

INTRODUCTION

HPTLC is a well known and versatile separation method which is type of planar chromatography, involves principle of adsorption. It is a flexible enough to analyze a wide variety of samples. It is useful in many ways as it is simple to handle and requires short analysis time to analyze the simple or complex samples. Nowadays, HPTLC serves as a preferred analytical tool for quantitative analysis of drug substances in bulk, from their formulations, from biological matrix, analysis of herbal extracts and standardization of herbal drugs.

Zolpidem tartrate (Zol-T), chemically known as N, N, 6-Trimethyl-2-ptolyl-imidazo(1,2-a)pyridine-3-acetamide L-(+)-tartrate (Fig. 1) is a non-benzodiazepine hypnotic agent binds preferentially to one benzodiazepine receptor subtype ω-1 benzodiazepine-1. [1-2] Zol-T acts as a sleep inducer without anticonvulsant and muscle relaxant effects. [1-3]

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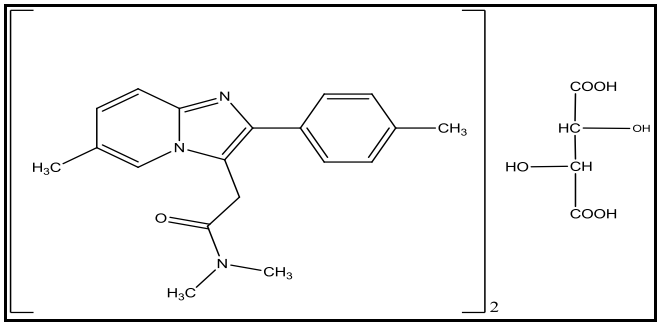

Fig. 1: Chemical structure of Zol-T

The hypnotic effect of Zol-T is same as that of drugs which are comes under benzodiazepine class, but it's structurally different from benzodiazepine and classified as imidazopyridine. [4] Zol-T is a white to off-white crystalline powder. It is sparingly soluble in water, alcohol, and propylene glycol.

Literature survey revealed that spectrophotometric [1, 3, 5-6], potentiometric [7] and high pressure liquid chromatographic (HPLC) methods [3-4, 8-14] have been developed for quantitative estimation of Zol-T. One HPTLC method has been reported by Zeany B., Moustafa A., *et al* for the determination of Zolpidem hemitartrate. [15] In referred scientific literature no HPTLC method has been found for the estimation of Zol-T.

The objective of research work was to develop accurate, precise, specific and economic analytical method for the estimation of Zol-T in bulk and marketed formulation. Considering the predefined objective of the research work, cost and suitability of analysis for estimation of Zol-T in bulk and its marketed formulation, HPTLC method was developed and validated as per the recommendations of ICH guidelines of analytical method validation.

Table 1 : Instrument and specifications of HPTLC

Sr. No.	Instrumentation details	Specifications
1	Make and model	Camag, Switzerland
2	Scanner	TLC scanner 5
3	Sampling mode	Manual with Linomat applicator
4	Syringe	Hamilton (100 μl)
5	Detection	Ultraviolet (UV) detector
6	Software	winCATS (ver.1.4.1.8)

MATERIALS AND METHODS

Materials and marketed formulation

Zol-T and its tablets were procured as generous gift sample for the purpose of academic research from local pharmaceutical company. Merck HPTLC aluminium plates precoated with silica gel 60 F_{254} were procured from local scientific and chemical supplier.

Reagents

Chemicals of (A.R. and HPLC grade) were purchased from S.D. Fine Chemicals, Mumbai, Maharashtra, India.

Instrumentation

Details of HPTLC instrument are given in Table 1.

Analytical Method Development (AMD)

Experimental

HPTLC method was developed for estimation of Zol-T. The detail of experimental work is presented in Table 2.

Table 2: Experimental procedures followed for HPTLC method development

Sr. No.	System/ Method/ Step	Procedure followed
1.	Preparation of sample and standard solution	Standard stock solution of Zol-T was prepared by dissolving 10 mg of drug in 10 ml methanol to obtain concentration 1000µg/ml (1000 ppm).
2.	Selection of stationary phase	Silica Gel 60 F_{254} precoated TLC plates were selected as chromatographic layer.
3.	Layer prewashing	Precoated TLC plates were prewashed with methanol to remove adsorbed material, impurities which include water vapours and other volatile substances from the atmosphere when they get exposed in the lab environment.
4.	Layer preconditioning	Prewashed plates were placed in oven at 100°C for 5 minutes prior to the sample application.
5.	Preparation of sample solution for estimation from marketed tablet formulation	Five tablets, each containing 10 mg of Zol-T were weighed and finely powdered. A quantity of powder equivalent to 10 mg of Zol-T was transferred to a 100 ml volumetric flask, dissolved in methanol and made the volume up to 100 ml with methanol. It was sonicated for 30 minutes in ultrasonication bath for complete dissolution of powdered drug if any. The solution was double filtered, first through 0.45µm whtaman filter paper and after that through 0.45 μm syringe filter in order to get cleared solution. Further, it was diluted with methanol to get the concentration of 50µg/ ml.
6.	Selection of detection wavelength	10µg/ml (10 ppm) solution of Zol-T was applied on HPTLC plate (suitable dimesion), scanned densitometrically over the range of 200- 700 nm using camag HPTLC scanner 5.
7.	Optimisation of chromatographic conditions	Many preliminary trials were carried out for selection and optimisation of, 1. Mobile phase composition 2. Chamber saturation time

AMD- Results and discussion

Selection of wavelength

UV absorption spectrum for 10 ppm solution of Zol-T (Fig. 2) was generated using camag HPTLC scanner 5 and winCATS (ver.1.4.1.8) software, 298nm wavelength was selected as a detection wavelength for chromatographic determination of Zol-T since at 298nm wavelength drug was showing maximum absorbance.

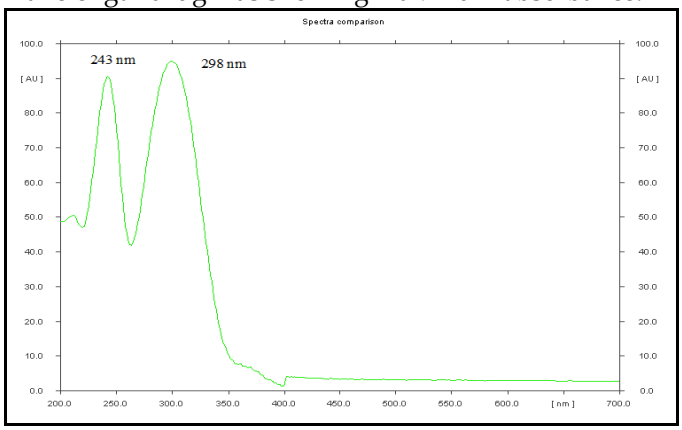

Fig. 2: HPTLC spectra of Zol-T

Optimization of chromatographic conditions

Based on literature survey [16-17], polarity and solubility of Zol-T many preliminary trials were carried out for selection of mobile phase composition, some are tabulated in Table 3.

Table 3: Trials for selection of mobile phase composition

Sr. No.	Mobile phase components	Composition (v/v/v)
1.	Diethylamine: methanol: ethyl acetate	(1: 4: 6)
2.	Ethyl acetate: chloroform: methanol	(4: 3: 3)
3.	Ethyl acetate: acetonitrile	(7: 3)
4.	Ethyl acetate: chloroform: acetonitrile	(4: 3: 3)
5.	Ethyl acetate: methanol: acetonitrile	(7: 1.5: 1.5)

Ethyl acetate: methanol: acetonitrile (7: 1.5: 1.5 v/v/v) was selected as optimised mobile phase composition. All optimized chromatographic conditions are tabulated in Table 4.

Table 4: Optimized chromatographic conditions

1.	**Mobile phase composition**	**Ethyl acetate: methanol: acetonitrile (7: 1.5: 1.5 v/v/v)**
2.	Detection wavelength	298 nm
3.	Chamber saturation time	30 min

Densitogram obtained using these optimised chromatographic conditions for Zol-T is shown in Fig. 3, R_f value for Zol-T was found to be 0.58.

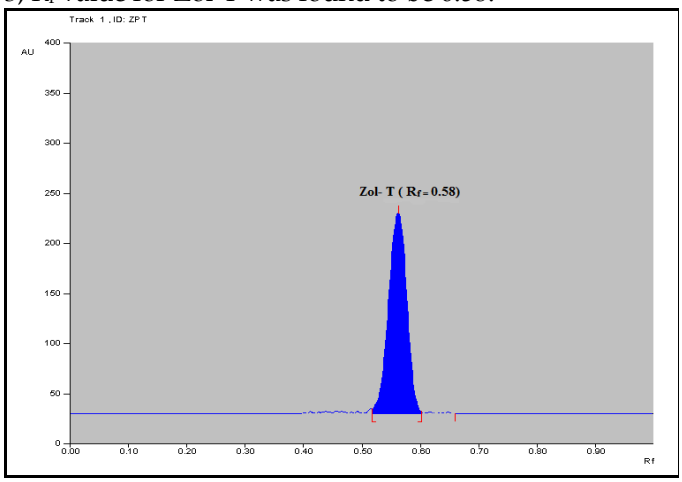

Fig. 3: Densitogram of Zol-T

ANALYTICAL METHOD VALIDATION (AMV)

Experimental

The developed HPTLC method was validated as per recommendations given by "ICH guidelines Q2(R1) for validation of analytical procedures: text and methodology". [18] Refer Table 5 for parameters and procedure followed for AMV.

Table 5: Analytical method validation: Parameters and procedure followed

S. No.	Parameters	Method/ Procedure followed
1.	**Linearity**	As per ICH, for the establishment of linearity, a minimum of 5 concentrations are recommended. A linear relationship was evaluated across the range of 200 to 800 ng/spot for Zol-T. It was obtained by plotting peak area against concentration of standard and finding regression coefficient (r^2).
2.	**Specificity**	As per ICH, specificity should be carried out to ensure identity of an analyte. The specificity of the method was determined by comparing the R_f value and densitogram of standard Zol-T with sample (tablet extract).
3.	**Precision**	Precision was carried out at two levels, as follows **Repeatability** Repeatability was assessed by using minimum of 9 determinations covering the specified range for the procedure (e.g., 3 concentrations/ 3 replicates each) **Intermediate Precision** Intermediate Precision was established to study the effects of random events i.e. days, on the precision of the analytical procedure. Intraday and interday precision studies were performed by taking 9 determinations of 3 concentrations/3 replicates each, at 3 different times in a same day and on 3 different days, respectively. Precision is reported as standard deviation and relative standard deviation (coefficient of variation) for each type of precision investigated.
4.	**Limit of Detection (LOD) and Limit of Quantification (LOQ)**	The values of Limit of Detection (LOD) and Limit of Quantitation (LOQ) were determined based on the standard deviation of the response and the slope of calibration graph. The quantitation was done with the help of following expression, LOD: $LOD = 3.3 \times \frac{\sigma}{S}$; LOQ: $LOQ = 10 \times \frac{\sigma}{S}$ σ = Standard deviation of response estimated based on the calibration curve. S = Slope of the calibration curve.
5.	**Accuracy**	Accuracy should be reported as percent recovery by the assay of known added amount of analyte in the sample. Accuracy should be assessed using a minimum of 9 determinations over a minimum of 3 concentration levels covering the specified range (e.g. 3 concentrations/3 replicates each of the total analytical procedure). In the present work percent recovery was calculated by performing recovery studies in triplicates of three concentration levels viz. 80%, 100%, 120% by adding known amount of standard solution of Zol-T. These samples were then analysed and the results obtained were compared with expected results.
6.	**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. For checking the robustness of the developed analytical method following parameters were deliberately changed, 1. Mobile phase composition 2. Chamber saturation time

AMV- Results and discussion

Linearity

Linear relationship was observed by plotting peak area against sample concentration. The calibration graph indicated that Zol-T produced a linear response across the range of 200-800 ng/ spot (Fig. 4). The linear regression data of calibration plot for Zol-T is given in Table 6.

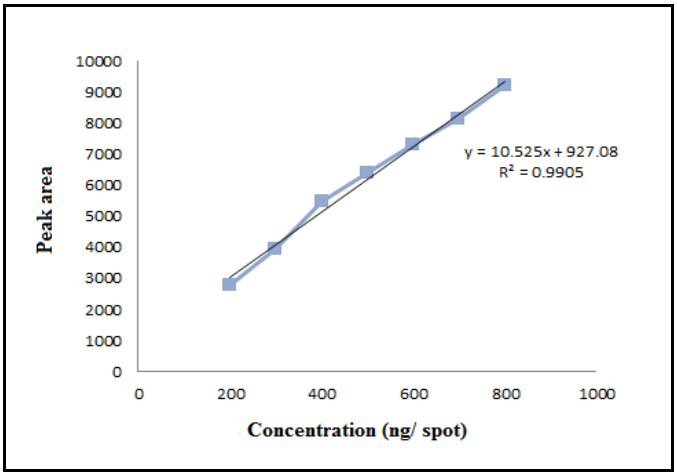

Fig. 4: Calibration plot for Zol-T

Table 6: Linear regression data of calibration plot for Zol-T

Sr. No.	Parameter	Results
1.	Range	200- 800 ng/spot
2.	r^2	0.9905
3.	y- intercept	927.08
4.	Slope	10.525

Specificity

When the densitogram of standard Zol-T was overlayed with the densitogram of sample (tablet extract) it was observed that the densitogram of Zol-T was exactly matching with the densitogram of tablet extract as shown in Fig. 5. Therefore the method is specific.

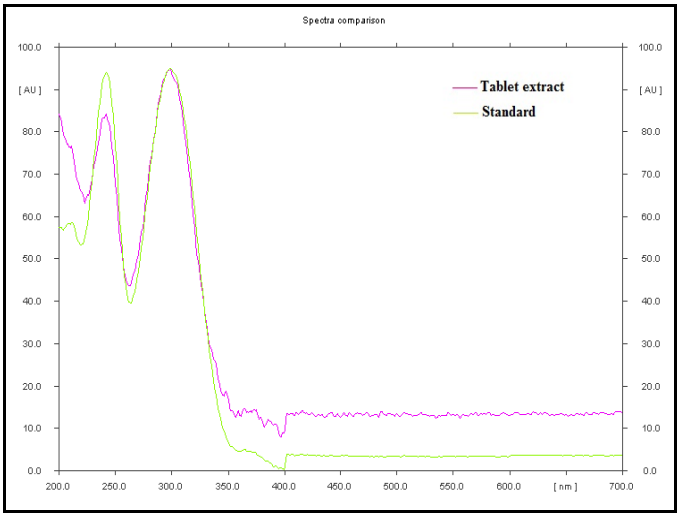

Fig. 5: Spectra of tablet extract and standard of Zol-T

Precision

Intra-day precision: It was performed at three different concentration levels low (300 ng/spot), mid (500 ng/spot) and high (700 ng/spot) respectively within the same day at three different times (session 1, 2, 3).

Inter-day precision: It was carried out at same concentration levels on three consecutive days, using same homogeneous sample. The % RSD values for both intra-day and inter-day precision were found within acceptable limit as shown in table 7 and 8 respectively.

Table 7: Intra-day precision studies

		Zol-T			Inference
Concentration levels		**Low**	**Mid**	**High**	
Concentration (ng/spot)		300	500	700	
Peak area	**Session 1**	3998.0	5924.5	8051.0	Acceptable % RSD, hence precise
	Session 2	3913.6	5845.5	8084.4	
	Session 3	3913.6	5842.9	8060.5	
Average peak area		3941.733	5870.967	8065.3	
Standard deviation		48.72836	46.37945	17.20959	
% RSD		1.236217	0.78998	0.213378	

Table 8: Inter-day precision studies

		Zol-T			Inference
Concentration levels		**Low**	**Mid**	**High**	
Concentration (ng/spot)		300	500	700	
Peak area	**Day 1**	4031.0	6240.3	8302.0	Acceptable % RSD, hence precise
	Day 2	4163.2	6259.0	8253.7	
	Day 3	4147.3	6146.4	8191.4	
Average peak area		4113.833	6215.233	8249.033	
Standard deviation		72.17495	60.34023	55.44748	
% RSD		1.754445	0.970844	0.672169	

Table 9: LOD and LOQ of Zol-T

Parameters	Readings obtained
LOD	16.99 ng/spot
LOQ	51.50 ng/spot

Limit of Detection (LOD) and Limit of Quantification (LOQ)

Values of LOD and LOQ calculated using slope of calibration plot for Zol-T is tabulated in Table 9.

Accuracy

Accuracy of the method is reported as percent recovery of known added amount of analyte in the sample. The accuracy of the method was established by performing recovery studies in triplicates of three concentration levels viz. 80%, 100%, 120% by adding known amount of Zol-T. Results obtained are given in Table 10.

Robustness

To determine robustness of analytical HPTLC method deliberate changes were made in the mobile phase composition and chamber saturation time. Effect of these changes on both the R_f values and peak areas were evaluated by calculating the relative standard deviations (%RSD). The results obtained are tabulated in Table 11.

Table 10: Accuracy- recovery studies

Drug	Level of percentage recovery (%)	Amount present in extract (ng/spot)	Amount added (ng/spot)	Total amount (ng/spot)	% recovery	Average % recovery	% RSD	Inferences
Zol-T	80	500	400	900	98.79	100.06	0.148661	Acceptable recovery hence accurate
	100	500	500	1000	101.04		1.014251	
	120	500	600	1100	100.37		1.603	

Table 11: Robustness results

Robustness parameters	Parameters changed	%RSD of area
Mobile phase composition (v/v/v)	Ethyl acetate: methanol: acetonitrile (7.2: 1.3: 1.5 v/v/v)	0.4738
	Ethyl acetate: methanol: acetonitrile (6.8: 1.7: 1.5 v/v/v)	1.6842
Chamber saturation time (minutes)	+ 2	0.9294
	- 2	1.8051

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