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Original article

Comparative UV-Spectroscopy and HPLC Methods for Content Analysis of Zolpidem Tartrate in Solid Dosage Forms

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A high performance liquid chromatography (HPLC) and ultra-violet spectroscopic (UV) methods were developed and validated for the quantitative estimation of Zolpidem tartrate in solid dosage forms. HPLC method was carried out using reverse phase technique on RP-18 column with a mobile phase composed of ammonium acetate buffer pH 5.0 and methanol (30:70, v/v) at ambient temperature. The mobile phase was pumped at a flow rate of 0.5 mL/min and detection was made at 243 nm with UV detector. UV method was performed with λ max at 243 nm with apparent molar absorptivity of 1279.25 L/mol.cm. Both the methods showed good linearity, recovery and precision. No spectra or chromatographic interferences from the tablet excipients were found in UV and HPLC methods. The results of analysis were validated according to ICH guidelines. Statistical comparison was done by student's t-test and F-test, which showed no significant difference between the results of both methods. So the proposed methods could be applicable for routine analysis of Zolpidem tartarate in bulk and pharmaceutical formulation.

Key words: Zolpidem tartrate, Validation, Comparative studies, Student's t-test and F-test

Zolpidem Tartarat'ın Katı Dozaj Formlarının Analizinde Karşılaştırılmalı UV Spektroskopisi ve YPSK Metotları

Zolpidem tartarat'ın katı dozaj formlarının kantitatif analizi için yüksek performanslı sıvı kromatografisi ve UV spektroskopisi yöntemi geliştirilmiş ve valide edilmiştir. YPSK metodu pH 5.0 amonyum asetat tampon-metanol (30:7,v/v) mobil fazı ile RP-18 kolonu kullanılarak ters faz tekniğiyle gerçekleştirilmiştir. 243 nm'de UV dedektör kullanılarak gerçekleştirilen yöntemde mobil fazın akış hızı 0.5 mL/dk'dır. UV yöntemi λ max=243 nm'de 1279.25 L/mol.cm molar absorbtivite değeri ile gerçekleştirilmiştir. Her iki yöntem de iyi bir doğrusallık , geri kazanım ve hassasiyet göstermiştir. UV ve YPSK yöntemlerinde tabletteki yardımcı maddelerinin spektral veya kromatografik girişimi söz konusu olmamıştır. Analiz sonuçları ICH kılavuzlara uygun olarak değerlendirilmiştir. İstatistiksel karşılaştırma Student t- ve F testleri ile yapılmış ve her iki yöntemin sonuçları arasında anlamlı bir farklılık görülmemiştir. Bu nedenle önerilen metodun ham halde ve farmasötik formülasyonda Zolpidem tartarat'ın rutin analizleri için kullanılabileceği anlaşılmıştır.

Anahtar kelimeler: Zolpidem tartarat, Validasyon, Karşılaştırmalı çalışma, Student t-testi ve F-testi

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INTRODUCTION

Zolpidem tartrate (ZT) is chemically N, N, 6-trimethyl-2-p-tolyl-imidazo [1,2- a]pyridine-3-acetamide L-(+)-tartrate (Figure 1). ZT is a non-benzodiazepine hypnotic of the imidazopyridine class and is available in 5 mg and 10 mg strength tablets for oral administration (1).

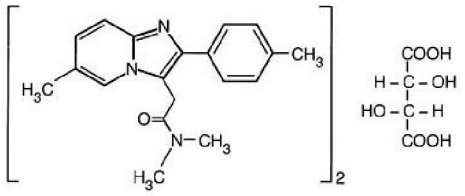

Figure 1. Chemical structure of zolpidem

ZT behaves as a sleep inducer without the muscle relaxant and anticonvulsant effects of the benzodiazepines. It is a non benzodiazepine hypnotic agent binds preferentially to one benzodiazepine receptor subtype ω-1 bezodiazepine-1thought to mediate hypnotic effects. The hypnotic actions of ZT, like benzodiazepine hypnotics, are mediated at the benzodiazepine recognition site of the GABAA receptor complex. However, the neuropharmacological profile of ZT is somewhat different from that of most benzodiazepines (2).

Literature survey revealed that, ZT was estimated by few RP-HPLC methods in dosage forms (3,4) and in biological samples (5-12). Detection was achieved by capillary electrophoresis (13) and radioimmunoassay (14). Some gas chromatographic methods (15-17) and spectroscopic methods (18,19) also have been reported for the estimation of ZT. In these reported methods, LC/MS, GC, and Radioimmunoassay methods were very expensive and were not used nowadays for routine analysis of drugs in dosage forms and derivative procedure for GC also difficult to achieve.

The main aim of the present work was to develop simple, fast and reproducible isocratic reverse phase HPLC, UV methods for the content analysis of ZT in bulk and pharmaceutical dosage forms. The results of the proposed methods were found to be satisfactory and reliable.

MATERIALS AND METHODS

Instrumentation and chromatographic condition

Chromatographic separation was performed on a Waters HPLC system equipped with a2487 series diode array detectors and LC solution software version 1.2. The study was achieved using Symmetry C18 column (5µm, 4.6X150mm, Xterra) at ambient temperature and the peak eluted with mobile phase consisting of ammonium acetate buffer pH 5.0: methanol (30:70,v/v) at a flow rate of 0.5 mL/min. the detection was made at 243 nm. The mobile phase was prepared daily, filtered through 0.45 µm nylon filter and degassed in sonicator prior to use. UV method was performed on Double beam UV-Visible spectrophotometer (Lab India UV 3000) with the λ max at 243 nm using 10mm matched quatz cells.

Chemicals

HPLC grade methanol, ammonium acetate (AR grade) and glacial acetic acid (AR grade) were purchased from Rankem Fine Chemicals Ltd. Water (HPLC grade) was obtained from a Milli-Q water purification system (Millipore, Milford, USA). ZT reference standard was kindly supplied by Cadila Laboratories, India.

The tablets (T.Ambien (Brand A) and T.Zolfresh (Brand B)) containing 10 mg of ZT were procured commercially.

Standard solution

For HPLC and UV: Accurately weighed 100 mg of ZT reference standard was transferred to 100 mL volumetric flask, dissolved and made up to the mark with mobile phase (1mg/mL concentration). The concentrations of 10-50 µg/mL were prepared individually from the above stock solution using mobile phase.

Sample solution

For HPLC and UV: Twenty tablets, each containing 10 mg of ZT was weighed and finely powdered. A quantity of powder equivalent to 10 mg of ZT was transferred to a 10 mL flask and made the volume up to the mark with mobile phase. It was sonicated for 30 minutes and filtered through 0.45 nylon filter. Further, it was diluted with same to get the concentration of 30 µg/mL.

Method validation

The method was validated for linearity, precision, recovery and system suitability. The objective of the method validation is to demonstrate that the method is suitable for intended use, as it is stated in ICH guidelines (20). Student's t-test and F-test were used to compare and verify the results.

Linearity: The standard calibration curves for UV and HPLC methods were obtained with series of concentrations of standard solutions. The solutions were prepared in triplicate and linearity was evaluated by linear regression analysis. LOD and LOQ were determined on the basis of slope and intercept values from regression equation.

Precision: The precision of the assay was studied with respect to both repeatability and intermediate precision. Repeatability was calculated from five replicate injections of freshly prepared ZT solution in the same equipment at a concentration of 30 µg/mL on the same day. The experiment was repeated by assaying freshly prepared solution at the same concentration additionally on four consecutive days to determine intermediate precision.

Accuracy: Percentage recovery was analyzed by taking the reference drug of ZT at three different levels, using three preparations at each level. The results were expressed as the percentage of ZT recovered in the sample and %RSD.

Specificity: Specificity of the HPLC method was assessed by comparing the chromatograms obtained from standard and sample preparations with those obtained from excipients which take part in the commercial tablet preparation.

Robustness: The robustness of the HPLC method was determined by analysis of samples under various conditions like changes in the percentage of organic phase (±10 %) in the mobile phase and changes in the flow rate (0.4 mL/min – 0.6 mL/min). The effect of retention time and system suitability parameters was observed. For UV, the drug content was analysed under the experimental variables like changes in the composition of the reagent and stability of the sample solution.

RESULTS AND DISCUSSION

HPLC method

A reversed phase HPLC method was developed to analyse the ZT in pharmaceutical preparation as a suitable method. The chromatographic conditions were adjusted in order to provide a better result for assay. Based on the solubility and pka value of the ZT, mobile phase (ammonium acetate buffer pH 5.0: methanol (30:70,v/v)) was selected which was

optimized from peak parameters like tailing factor, number of theoretical plates, run time, easy of preparation and cost. The mixture of 50:50, v/v ratio of aqueous and organic phase has shown the peak at the retention time of 16.0 min. It was reduced by gradual increase of organic phase composition in the mobile phase to get the individual peak nearly at 5.0 min. A typical chromatogram (Figure 2) was obtained with retention time of 4.9 min which showed a rapid determination of drug for frequent analysis.

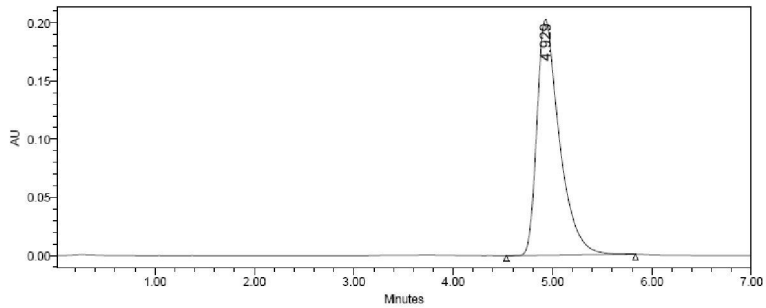

Figure 2. Chromatogram for standard zolpidem tartrate (30 µg/mL)

In this proposed method, the peak was eluted with a tailing factor (T) 1.6 and number of theoretical plates (N) 2282. From the calibration curve (Figure 3), ZT showed good linearity in the range of 10-50 µg/mL with regression equation of y=104746x + 28930 and correlation co-efficient of r^2= 0.9999, indicating a high sensitivity of the method (Table 1).

The precision of the method was ascertained by repeatability (*Intra-day*) and intermediate precision (*Inter-day*) the results of % purity and % RSD were tabulated in Table 2 which showed good precision. The recovery of the drug was achieved by taking and analyzing different levels (50%, 100 % and 150 %) of standard drug in similar manner. The results of mean percentage recovery, % RSD and standard error were showed in Table 3.

There was no significant change in the system suitability factors of ZT when the organic composition and flow rate were changed. The low values of the % RSD indicated that the

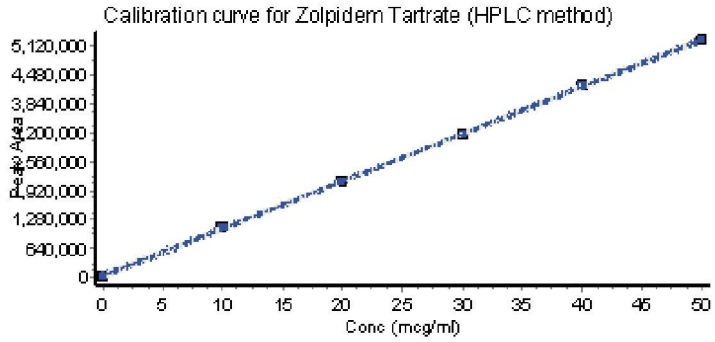

Figure 3. Standard calibration curve for HPLC method

method was robust enough and the results were tabulated in Table 4.

Table 1. Results for linearity study

Parameters	HPLC method	UV method
Linearity Range (µg/mL)	10-50	10-50
LOD (µg/mL)	0.0165	0.1118
LOQ (µg/mL)	0.0555	0.3389
Slope	104746	0.01608
Standard Error on Slope	631.56	0.000199
Confidence Limit of Slope	102992 to 106499	0.01553 to 0.01663
Intercept	28930	0.01033
Standard Error on Intercept	19122	0.006035
Confidence Limit of Intercept	-24151 to 82011	-0.006420 to 0.02709
Correlation Co-efficient	0.9999	0.9994
Standard deviation of Residuals	26420	0.008339

LOD = Limit of Detection, LOQ = Limit of Quantitation

UV method

The spectrum of 30 µg/mL (Figure 4 & 5) of ZT standard solution and sample solution showed intense absorbance peak with λ max at 243 nm.

Table 2. Results for precision study data

Precision parameters	HPLC method		UV method	
	Peak Area*	%RSD*	Absorbance*	%RSD*
Repeatability	3126579	1.28	0.496	0.168
Intermediate Precision	3214112	1.26	0.483	0.313

*(n=5)

The good linearity (Figure 6) was obtained on standard solutions of ZT over the concentration range of 10-50 µg/mL. The precision of the method was assessed with %RSD values of 0.168 for repeatability and 0.313 for intermediate precision which were found for five replicates at a concentration of 30 µg/mL (Table 2). Accuracy of the method was done as per HPLC method and the mean percentage content was obtained always between 99.60 % and 100.50 % with 95 % confidence interval of 98.21 and 102.16 (Table 3).

Robustness of the method was assessed by evaluating the influence of small variations of experimental variables like changes in the composition of the reagent and stability of the sample solution on the analytical performance. The small variations in any of the variables did not significantly affect the results. This provided an indication for the reliability of the proposed method during routine analysis (Table 4).

Table 3. Results for recovery study data

Brands	Levels of drug taken	Mean percent recovery*	% RSD*	S.E	95 % CI
		HPLC method			
Brand A	50 %	100.41	0.7453	0.4321	98.55-102.27
	100 %	101.10	0.2123	0.1239	100.57-101.64
	150 %	101.00	1.3733	0.8009	97.55-104.44
Brand B	50 %	100.20	0.4574	0.2646	99.06-101.34
	100 %	100.19	0.2908	0.1683	99.46-100.92
	150 %	99.81	0.5814	0.3350	98.36-101.25
		UV method			
Brand A	50 %	100.45	0.4781	0.0762	100.12-100.77
	100 %	99.83	0.5140	0.2963	98.55-101.11
	150 %	100.19	07937	0.4591	98.21-102.16
Brand B	50 %	100.12	0.6231	0.3602	98.57-101.67
	100 %	99.73	0.5522	0.3180	98.36-101.10
	150 %	99.63	0.3225	0.1856	98.83-100.43

*(n=3), % RSD = Percentage Relative Standard Deviation, S.E = Standard Error, 95 % CI = 95 Percent Confidence Interval

Table 4. Data for robustness study

	Parameters		USP tailing factor	Theoretical plates	% content
HPLC	Organic phase composition (%)	60	1.6	2325	100.54
		80	1.6	2369	99.94
	Flow rate (mL/min)	0.4	1.6	2483	100.21
		0.6	1.6	2298	100.48
UV	Composition of reagents in solvent	20:80	-	-	99.68
		40:60			99.43
	Stability of sample solution	After 2 h	-	-	98.67
		After 4 h			97.48

% content = Percentage Drug Content

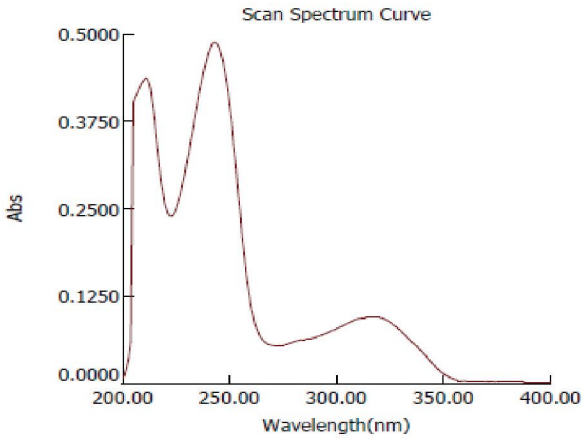

Figure 4. UV spectrum for zolpidem tartrate (Standard)

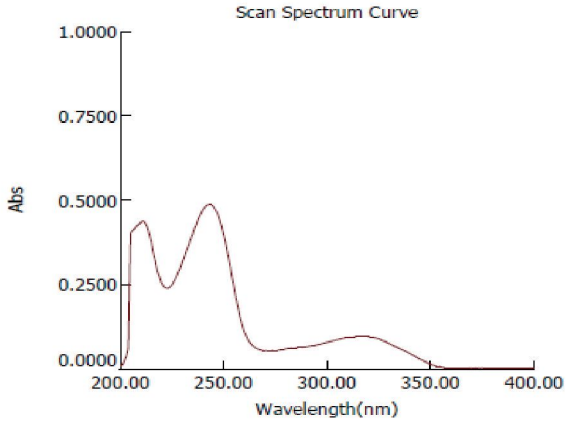

Figure 5. UV spectrum of zolpidem tartrate (Sample)

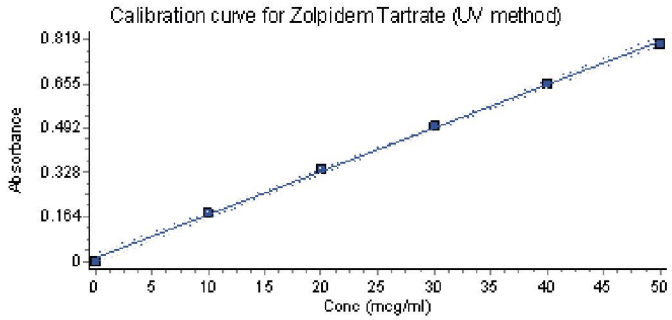

Figure 6. Standard calibration curve for UV method

Table 5. Data for statistical comparison results of UV and HPLC methods

Brands	Level of drug taken (%)	% RSD for % content		*F*-test[a]	*t*-test[b]
		HPLC	UV		
Brand A	50	0.7453	0.4781	2.428	0.071
	100	0.2123	0.5140	5.720	3.955
	150	1.3733	0.7937	3.043	0.877
Brand B	50	0.4574	0.6231	1.653	0.704
	100	0.2908	0.5522.	3.571	1.279
	150	0.5814	0.3225	3.259	0.470

[a,b] Limits of 95 % confidence Interval

Statistical comparison of the proposed methods

From the validation results, the above said methods were ascertained as suitable for routine quality control analysis of ZT in marketed formulations. They were applied for two different brands of ZT and %RSD for percentage recovery of each level (50%, 100% and 150%) were statistically compared (Table 5). Student's *t*-test and *F*-test were applied and revealed that no significant difference between the experimental values obtained during analysis by two methods. The calculated t-value and F-value were found to be less than the tabulated values of both the methods at 95 % confidence interval. From this report, it was evident that the proposed UV and HPLC methods were applicable to the ZT in tablets in convenient manner.

CONCLUSION

The HPLC method and UV spectroscopy method developed and validated for the analysis of ZT tablets were found to be reliable, simple, fast, accurate and precise. The results of UV method showed no significant difference from the HPLC method. The purpose to develop the new spectroscopic method is not to replace the available methods for the content analysis of ZT in tablet dosage forms, but to use as an alternative method where the advanced instruments like HPLC, GC, and RIA are not available for routine analysis. Hence, it was concluded that, there is no need of extraction procedure for sample preparation and less time consuming. The spectroscopy method requires only the wavelength scan, so it can be utilized for frequent analysis ZT in pharmaceutical dosage forms than the other sophisticated methods.

REFERENCES

1. Tripathi KD, Essentials of medical pharmacology. 5th ed., pp. 357 and 366, Jaypee Brothers Medical Publishers (P) Ltd, 2003.
2. Budavari S, The Merck Index. 12th ed., pp. 10322, White house Station (NJ): Merck and Co Inc, 1996.
3. Laviana L, Mangas C, Fernández-Marí F, Bayod M, Blanco D, Determination and in-process control of zolpidem synthesis by high-performance liquid chromatography, J Pharm Biomed Anal 36(4), 925 - 928, 2004.

4. Maharajan MP, Sawant SD, Validated RP-HPLC method for the determination of Zolpidem tartrate in bulk and tablet dosage forms, J Pharm Res 4(10), 3789 -3791, 2011.
5. Ring PR, Bostick JM, Validation of a method for the determination of zolpidem in human plasma using LC with fluorescence detection, J Pharm Biomed Anal 22(3), 495 - 504, 2000.
6. Guinebault P, Dubruc C, Hermann P, Thénot JP, High-performance liquid chromatographic determination of zolpidem, a new sleep inducer, in biological fluids with fluorimetric detection, J Chromatogr B 383(1), 206 – 211, 1986.
7. Nirogi RVS, Kandikere VN, Shrivasthava W, Mudigonda K, Quantification of zolpidem in human plasma by high-performance liquid chromatography with flourescence detection, Biomed Chromatogr 20(10), 1103 – 1108, 2006.
8. Durol ALB, Greenblatt DJ, Analysis of zolpidem in human plasma by high-performance liquid chromatography with fluorescence detection: application to single-dose pharmacokinetic studies, J Anal Toxicol 21(5), 388 – 292, 1997.
9. Wang Q, Sun L, Lau CE, Determination of zolpidem in serum microsamples by high-performance liquid chromatography and its application to pharmacokinetics in rats, J Chromatogr B Biomed Sci Appl 734(2), 299–305, 1999.
10. Ptacek P, Macek J, Klima J, Rapid and simple method for the determination of zolpidem in human plasma by high-performance liquid chromatography, J Chromatogr B Biomed Sci Appl 694(2), 409 – 413, 1997.
11. Ascalone V, Flaminio L, Guinebault P, Thenot JP, Morselli PL, Determination of zolpidem, a new sleep-inducing agent, and its metabolites in biological fluids: pharmacokinetics, drug metabolism and overdosing investigations in humans, J Chromatogr B Biomed Sci Appl 581(2), 237 – 250, 1992.
12. Kratzsch C, Tenberken O, Peters FT, Weber AA, Kraemer T, Maurer HH, Screening, library-assisted identification and validated quantification of 23 benzodiazepines, flumazenil, zaleplone, zolpidem and zopiclone in plasma by liquid chromatography/mass spectrometry with atmospheric pressure chemical ionization, J Mass Spectrom 39(8), 856 – 872, 2004.
13. Hempe GL, Blanschke G, Direct determination of zolpidem and its main metabolites in urine using capillary electrophoresis with laser-induced fluorescence detection, J Chromatogr B Biomed Sci Appl 675(1), 131 - 137, 1996.
14. De Clerck I, Daenens P, Development of a radioimmunoassay for the determination of zolpidem in biological samples, Analyst 122(10), 1119 – 1124, 1997.
15. Stanke F, Jourdil N, Bessard J, Bessard G, Simultaneous determination of zolpidem and zopiclone in human plasma by gas chromatography-nitrogen-phosphorus detection, J Chromatogr B Biomed Sci Appl 675(1), 43 - 51, 1996.
16. Gaillard Y, Gay-Montchamp JP, Ollagnier M, Simultaneous screening and quantitation of alpidem, zolpidem, buspirone and benzodiazepines by dual-channel gas chromatography using electron-capture and nitrogen-phosphorus detection after solid-phase extraction, J Chromatogr B Biomed Sci Appl 622(2), 197 - 208, 1993.
17. Debruyne D, Lacotte J, Hurault de Ligny B, Moulin M, Determination of zolpidem and zopiclone in serum by capillary column gas chromatography, J Pharm Sci 80(1), 71 - 74, 1991.
18. Patil KS, Pore YV, Bhise SB, Spectrophotometric Estimation of Zolpidem in Tablets, J Pharm Sci Res 2(1), 1 - 4, 2010.
19. Rajiv C, Kumar A, Goyal A, Spectrphotometric methods for determination of zolpidem tartrate in tablet formulation, J Pharm Bioallied Sci. 2(4), 365 - 368, 2010.
20. International Conference on Harmonization of Technical Requirements for the Registration of Pharmaceutical for Human Use: Validation of Analytical Procedure, Text and Methodology, Q2(R1), 2005.

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