IJPSR (2026), Volume 17, Issue 7 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 03 March 2026; received in revised form, 09 June 2026; accepted, 19 June 2026; published 01 July 2026

QUANTITATIVE BIOANALYTICAL DERIVATIVE SPECTROSCOPIC STUDIES OF ZOLPIDEM TARTRATE NANOSPHERES

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Keywords:

Zolpidem tartrate Nano spheres, XRD, Zeta analysis, Zero and first order kinetics, AUC, Human plasma spiking studies, and pharmacokinetic studies

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ABSTRACT: We developed a quantitative, accurate, and cost-effective ultraviolet (UV) spectrophotometric method to estimate zolpidem tartrate nanospheres. These nanospheres were prepared using the solvent evaporation method. X-ray diffraction (XRD) analysis showed peak heights at 19.914, 23.0138, and 40.5246, with values of 225.20, 61.58, and 29.25. Scanning electron microscopy (SEM) confirmed their morphology at magnifications of 50× and 2× µm. Zeta analysis showed a Z- average of 2234d:nm, a peak at 245.6nm, and a zeta potential of – 14.0mV. The nanospheres had an absorbance at 201 nm using the zero-order spectrum method. First-order derivative spectra showed a maximum at 305 nm and a minimum at 280 nm, with absorbance values of 0.9133 and 0.8041. In method I, using bromocresol green, the nanospheres had an absorbance maximum of 0.426 at 429.6 nm and a minimum of 0.786 at 202.2 nm. The area under the curve (AUC) was calculated from 329 nm to 415 nm, with a linearity range of 5-30 µg/mL and R2 = 0.9869. In method II, with bromophenol blue, absorbance maxima were 0.421 at 367.2 nm and 0.886 at 235 nm. The AUC ranged from 279 nm to 329 nm, and linearity was observed from 5 to 30 µg/mL with R2 = 0.9787. In method III, using Sudan Red–III, absorbance maxima were 1.934 at 372.6 nm and 1.3003 at 219.3 nm, with R2 = 0.9894. The AUC was from 275 nm to 330 nm. Validation in human plasma showed linearity from 100 µg/mL to 500 µg/mL, with R2 = 0.9684. Pharmacokinetic studies found a C_{max} of 357.274 ± 32.57 ng/mL, T_{max} of 0.8333 hours, AUC_{0-t} of 623.32 ± 68.55, and $AUC_{0-\alpha}$ of 656.013 ± 358.640.

INTRODUCTION: Nanotechnology is a revolutionary path for technological development, which concerns the management of drugs at the nanometer scale [1]. Zolpidem tartrate is used for the treatment of insomnia, which is classified under BCS class I (Z- category) drug [2].

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DOI: 10.13040/IJPSR.0975-8232.17(7).2089-97

This article can be accessed online on www.ijpsr.com

DOI link: https://doi.org/10.13040/IJPSR.0975-8232.17(7).2089-97

Zolpidem tartrate, chemically known as (2R,3R)-2,3-dihydroxybutanedioic acid; bis(N,N-dimethyl-2-[6-methyl-2-(4-methylphenyl) imidazo [1, 2-a] pyridin-3-yl]acetamide). Zolpidem tartrate binds to α5–containing GABAA receptor subtypes with low affinity. Perusal of literature reveals that Zolpidem tartrate was determined by liquid chromatographic methods in biological fluids [3].

Spectroscopic methods, such as UV-Visible and fluorometric, were developed for Zolpidem tartrate. However, the formulation of zolpidem nanospheres was not reported. In the present study, we

attempted to formulate Zolpidem tartrate nanospheres and developed three simple spectrophotometric methods using three indicators (bromocresol green, bromophenol blue, and Sudan red–III) we determined pharmacokinetic parameters (Cmax and Tmax) in biological fluids.

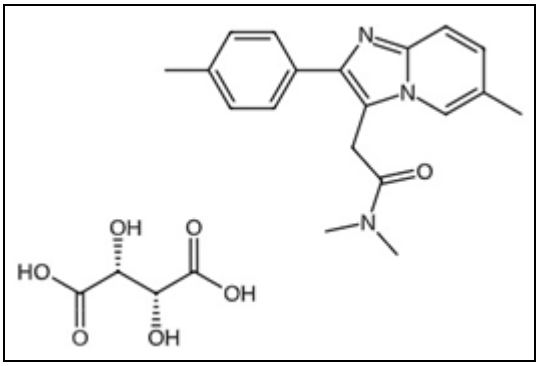

FIG. 1: ZOLPIDEM TARTRATE

MATERIALS AND METHODS:

Materials: Zolpidem tartrate tablets were procured from the local market in Kootanadu, Kerala, and the active pharmaceutical ingredient (API) was extracted using iodobenzene/hexane. Acetonitrile, Methanol, and sulphuric acid were procured from Prowess Chemicals, Ernakulam town, Kerala.

Solubility Studies [4]: The solubility of Zolpidem tartrate is an important property because it affects how well the drug can be absorbed by the body. To study its saturation solubility, 10 mg of Zolpidem was added to 250 mL flasks containing different solvents. These solvents included water, alcohol, 0.1 N hydrochloric acid, 0.1 N sulphuric acid, pH 6.8 phosphate buffer, pH 4.5 acetate buffer, methanol, and acetonitrile. After reaching saturation, the solutions were collected, filtered using a 0.45 µm membrane filter, and then concentrated under reduced pressure.

Formulation of Zolpidem Tartrate Nanospheres (ZPNS) by Solvent Evaporation Method [5]: 100 mg of Poly Lactic-co-Glycolic acid (PLGA 50:50) with a molecular weight of 30 kDa, sourced from Mitsui Chemicals, New Delhi. This was dissolved in 10 mL of dichloromethane and stirred at 1000 rpm and 37°C until clear. Next, 10 mg of zolpidem tartrate (API) was added to the polymer solution, and the mixture was stirred at the same speed to form a primary emulsion, maintaining a 1:1 drug-to-polymer ratio. Then, 25 mL of polyvinyl alcohol (PVA) was added, and the mixture was homogenised at 1000 rpm and 37°C for 12 hours. The resulting dispersion was centrifuged at 20,000 rpm for 30 minutes. The supernatant and excess dichloromethane were removed using a rotavap flash evaporator. The PVA concentration was measured at 6.8%. The yield of zolpidem nanospheres was 0.0062 g (6.2 mg) **Fig. 1**. Drug loading (DL%) and encapsulation efficiency (EE%) were 1.55% and 0.38%, respectively. The polydispersion index (PDI) for zolpidem tartrate nanospheres was 0.569. The emulsion was then analysed for morphological characteristics using a scanning electron microscope (SEM) at magnifications of 300× and 2× respectively.

Particle size Characterisation [5]: The lyophilised nanospheres were suspended in deionised water. We measured particle size, size distribution, polydispersity index, and Zeta potential using a Nano-Zeta sizer (Alvern, Westborough, USA). The Zeta potential was –14.0, and the Z-average diameter was 2234 d.nm (Fig. 2-3).

Calibration Studies of Zolpidem Tartrate Nanospheres (ZPNS) [6]: To prepare the stock solution (0.005 mg/mL or 50 µg/mL) of Zolpidem tartrate nanospheres, 0.5 mg of the nanospheres is dissolved in 10 mL of 0.1 N sulphuric acid. The solution is then degassed for 15 minutes. Part of this stock solution is stepwise diluted with 0.1 N sulphuric acid to prepare working standard solutions with concentrations ranging from 5 to 30 µg/mL. These dilutions are scanned from 200 to 400 nm using 0.1 N sulphuric acid as the blank, and the results were shown in **Table 3.**

FT-IR Studies [7]: In this study, we used the KBR disc method. The sample was thoroughly mixed with dry powdered KBR, then pressed into a transparent disc under high pressure using special dies. We placed the disc in an IR spectrometer and recorded the spectrum. The scanning range was 450-7000 $^{cm-1}$, with a resolution of 1 $^{cm-1}$. We aimed to examine the compatibility, structural integrity, and encapsulation efficiency of Zolpidem tartrate with polymers like PLGA 50:50 (Poly Lactic–Co–Glycolic acid) and polyvinyl alcohol. We compared and interpreted the spectra based on functional groups. The results are shown in **Table 1** and **Fig. 4.**

X-ray Characterization Studies of Zolpidem Tartrate Nanospheres (ZPNS) [8]: Zolpidem tartrate nanospheres were analysed for size characteristics using X-ray diffraction with an alumina coating. Diffraction data were collected from a parallel beam CU K ά bruker diffractometer. The results were analysed using a beta version of Bruker's Topas 2.1 software. The results were embodied in **Table 2** in **Fig. 5.**

Scanning Electron Microscopic Studies [9]: A 100 µg sample of zolpidem tartrate nanospheres was mounted on silver electrical tape and coated with gold in a neutral environment under reduced pressure using an SCD005 Baltek sputter coater. After that, a thin layer of conductive material was added to prevent charging.

A voltage between 1 and 10 KV was used to limit beam penetration and protect the sensitive zolpidem nanospheres. Secondary electron detectors were used for detection. The samples were viewed at different magnifications with a Joel 457-V SEM (Tokyo, Japan) and photographed **Fig. 6** & **7.**

Instrumentation: A double-beam UV–Visible spectrometer (Systronics-1500A) with a spectral bandwidth of 1.5 nm and automatic wavelength correction, along with a pair of 10 mm quartz cells, was employed for the experimental studies.

Method –I (Bromo Cresol Green) [10]: Aliquots of standard drug solutions (5–30 µg/mL) were added to a series of 10 mL volumetric flasks. A 100 µg/mL solution in 0.1 N H_2SO_4 was transferred and diluted with the same solvent to prepare zolpidem tartrate nanosphere dilutions at concentrations of 5–30 µg/mL. For each dilution, 5 mL was placed in a separating funnel, followed by the addition of 5 mL of 0.2% w/v bromocresol green and 5 mL chloroform (1:1).

The mixture was gently shaken for 5–10 minutes and then allowed to stand until the aqueous and chloroform layers separated. The chloroform layer was collected, and the corresponding zero-order, first-order, and area under the curve (AUC) values were determined. A calibration curve of zolpidem tartrate nanosphere measured absorbance was plotted in **Fig. 8**. Spectral characteristics are presented in **Table 4.**

Method-II (Bromophenol Blue): In method II, aliquots of a standard drug solution (100 µg/mL) in 0.1N H_2SO_4 were transferred into a series of 10 mL analytical flasks and diluted with the same solvent to obtain concentrations ranging from 5 to 30 µg/mL of zolpidem tartrate. For each dilution, 5 mL was placed in a separating funnel, followed by the addition of 5 mL of 0.2% w/v bromophenol blue and 5 mL of chloroform (1:1). The mixture was gently shaken for 5 to 10 minutes and then allowed to stand until the aqueous and chloroform layers separated. The chloroform layer was collected, and the corresponding zero-order, first-order, and area under the curve (AUC) values were determined. A calibration curve was constructed in **Fig. 9** by plotting the concentration of zolpidem tartrate nanospheres against the measured absorbance. Spectral characteristics are presented in **Table 4**.

Method- III (Sudan Red-III): For method III, aliquots of standard drug solution (100 µg/mL) in 0.1N H_2SO_4 were transferred into a series of 10 mL analytical flasks and diluted with the same solvent to obtain zolpidem tartrate concentrations ranging from 5 to 30 µg/mL. To 5 mL of each dilution placed in a separating funnel, 5 mL of 0.2% w/v Sudan –III and 5 mL of chloroform were added (1:1). The mixture was gently shaken for 5 to 10 minutes, after which the aqueous and chloroform layers were separated. The chloroform layer was collected, and zero-order, first-order, and area under the curve (AUC) values were determined. A calibration curve was constructed in **Fig. 10** by plotting the concentration of zolpidem tartrate nanospheres against the measured absorbance. Spectral characteristics are presented in **Table 4.**

Calibration of Zolpidem Tartrate Nano Spheres (ZPNS) Spiked in Human Plasma: [11]

Preparation of Human Plasma: Blood samples were obtained from six healthy volunteers with an average weight of 75 kg, following informed consent and ethical committee approval (No: KCP/2025/IEC/0005) from Mookambiga Medical College, Nagercoil, K.K. district, Tamil Nadu, India. Sample collection was conducted under the supervision of Dr. M. Jabel, Psychiatrist and Head of Department at Mookambiga Medical College. Blood samples were drawn at 0, 10, 20, 30, 40, 50, 60, 70, 80, and 90 minutes. Each sample was

placed in heparinised Vacutainer tubes and centrifuged at 30,000 rpm for 15 minutes. The resulting supernatant was collected and subjected to freeze-thawing at -10º C.

Preparation of Standards: Stock standard solutions of Zolpidem tartrate nanospheres (5 µg/mL) were prepared in methanol and stored at 4 °C. Standard solutions for spiking (100, 200, 300, 400, and 500 µg/mL) were obtained by diluting the stock solution with methanol. Long-term stability was assessed by storing triplicate low, medium, and high quality control (QC) samples at 37 °C for 24 hours.

Manual – Shaking Assisted Dispersive Liquid – Liquid Extraction: A 500 µL volume of human plasma was spiked with zolpidem tartrate nanospheres at varying concentrations (100–500 µg/mL). Subsequently, 600 µL of acetonitrile, serving as both a protein precipitant and a dispersive agent, was added. The mixture was vortexed, and the drug was extracted using methanol. The resulting drug sample was analysed by UV absorption to determine the λmax. Matrix effects were found to be 126.42%. Linearity data are presented in **Table 5** & **6** as well as **Fig. 10-12**. Pharmacokinetic parameters, including C_{max}, T_{max}, AUC0–t, and AUC0–α, were calculated for zolpidem tartrate nanospheres in the test formulations.

RESULTS AND DISCUSSION:

FT-IR studies of Zolpidem Tartrate Nano Spheres (ZPNS) by Solvent Evaporation Method: FT – IR studies revealed the presence of functional groups of Zolpidem tartrate (drug). Based on the report, there is no interference between the polymers and the drug during nanospheres formulation.

TABLE 1: FT-IR STUDIES OF ZOLPIDEM TARTRATE NANOSPHERES

S. no.	Functional group revealed in the IR spectra	Wavelength cm^{-1}
1.	C-H Stretching	3318.61
2.	CH_3 Stretching	2928.65
3.	C=O Stretching	1727.09
4.	N-H Stretching	1641.77

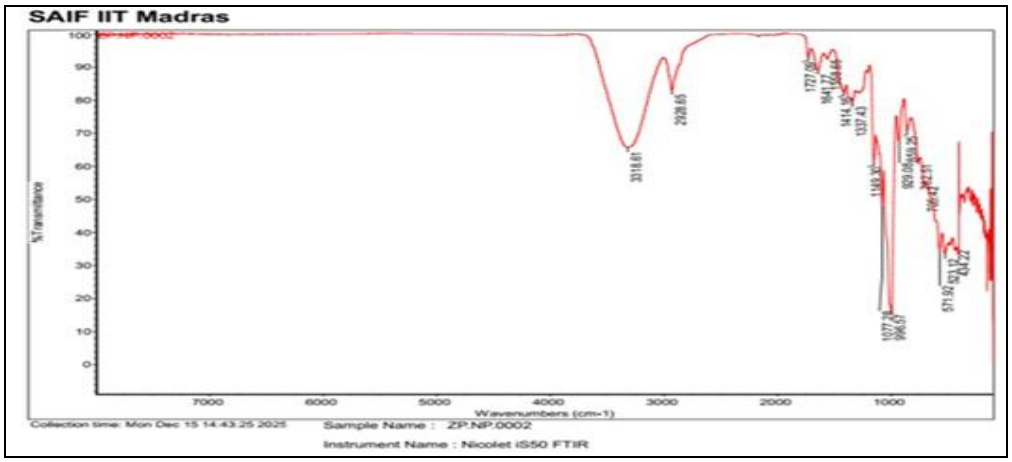

FIG. 2: FT –IR SPECTRA OF ZOLPIDEM TARTRATE NANOSPHERES

FIG. 3: FORMULATION OF ZOLPIDEM TARTRATE NANOSPHERES

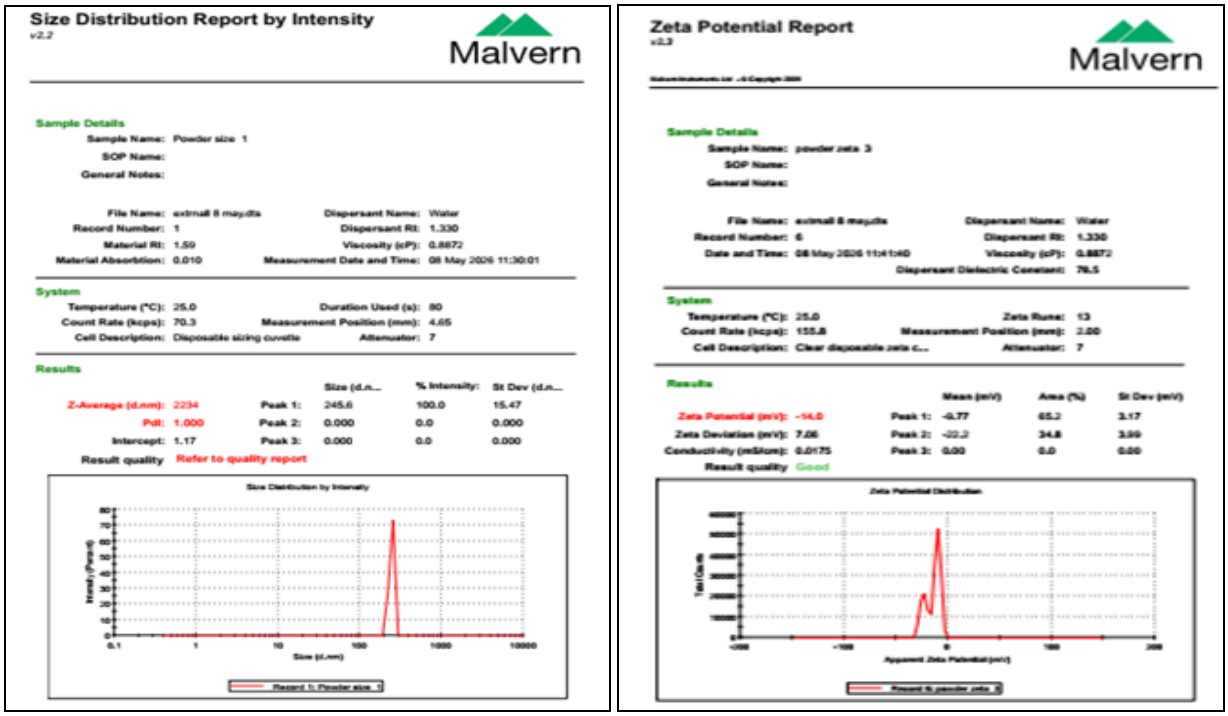

FIG. 4 & 5: PARTICLE SIZE DISTRIBUTION AND ZETA SIZE ANALYSIS OF ZOLPIDEM TARTRATE

X-ray Characterization of Zolpidem Nanoparticles: Zolpidem nanoparticles (nanocrystalline powders) were analysed for size characteristics using several different techniques with alumina coating. Diffraction data were collected using a Bruker Cu Kα diffractometer, and the results were embodied at brukers topas 2.1 software. The results obtained were tabulated in **Table 2** and shown in **Fig. 6.**

TABLE 2: X-RAY CHARACTERIZATION STUDIES OF ZOLPIDEM TARTATE NANOSPHERES (ZPNS)

S. no.	Scan Parameters	Values
1.	Start Position [20º]	10.0131
2.	End Position [20º]	89.9891
3.	Step Size [20º]	0.0260
4.	Scan time	Continuous
5.	Peak height (Pos [20º] at 19.914	225.20
6.	Peak height (Pos [20º] at 23.0138	61.58
7.	Peak height (Pos [20º] at 40.5246	29.25

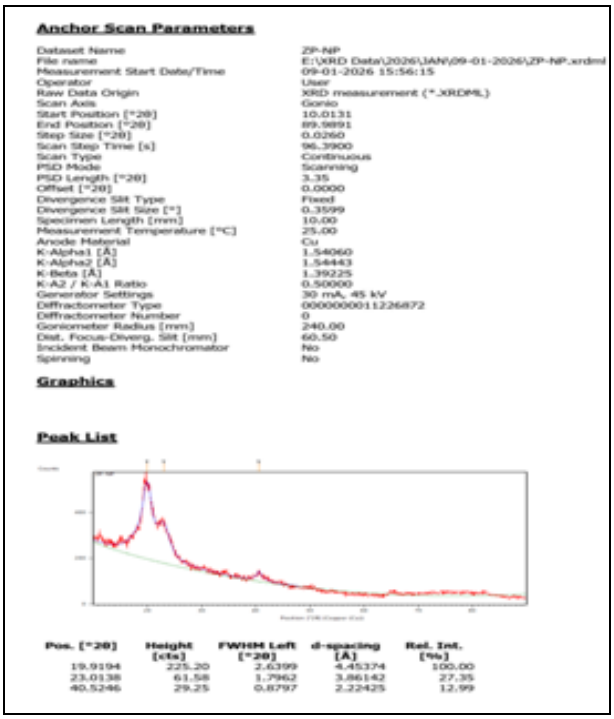

FIG. 6: XRD STUDIES OF ZOLPIDEM TARTRATE NANOSPHERES

SEM Studies of Zolpidem Tartrate Nanospheres: Zolpidem tartrate nanospheres are dispersed in a carbon tape and coated with a thin layer of conductive material, carbon and subjected to an accelerating voltage of 1-10 KV and magnified at 2-50nμm. Magnification unit 50μm (WD 12.1mm) and 2μm (WD 11.8mm).

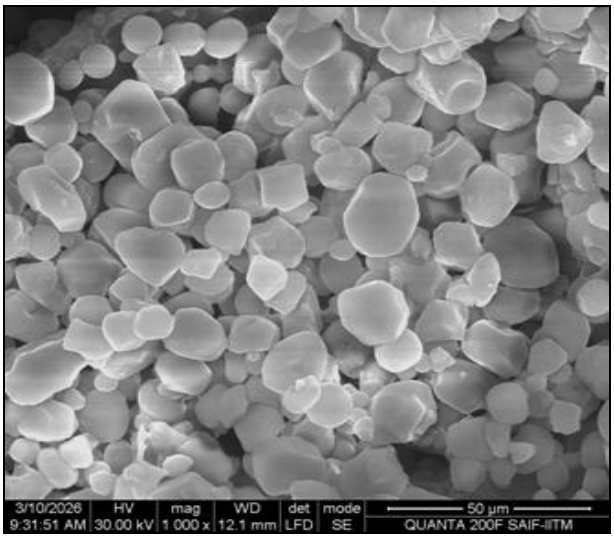

FIG. 7: SEM IMAGE OF ZOLPIDEM NANOSPHERES AT 50μM

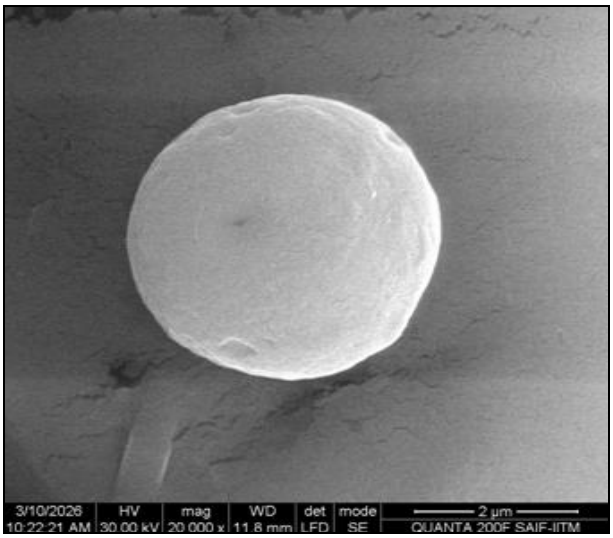

FIG. 8: SEM IMAGE OF ZOLPIDEM NANOSPHERES AT 2μM

Beer's law was obeyed in the concentration range of 0.05-20μg/mL for the methods I, II, and III, respectively, in **Table 3.** The linear regression equations were found to be R^2 = 0.989 (method–I), R^2 = 0.9787 (method-II), and R^2 = 0.9894 (method-III), respectively. AUC were found to be 329-415nm for method-I, 279-321nm for method-II, and 279-325nm for method-III, respectively **Table 3, Fig. 7-11**.

TABLE 3: SPECTRAL CHARACTERSTICS OF ZOLPIDEM TARTRATE NANOSPHERES

S. no.	Parameters	Method-I	Method-II	Method-III
1	Beer's –Lambert's limits (μg/mL)	0.075-1	0.073-1	0.077-1
2	ƛ max/Amplitude range (nm)	296	296	295.2
3	Correlation coefficient	0.9869	0.9787	0.9194
4	Slope (a)	0.087	0.089	0.090
5	Intercept (b)	0.006	0.007	0.007
6	AUC (Area Under Curve)	329-415nm	279-321nm	279-325 nm

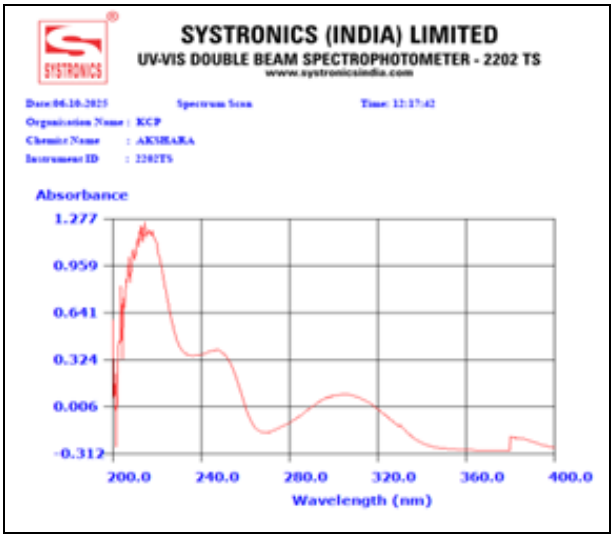

FIG. 9: ZERO ORDER SPECTRUM OF ZOLPIDEM TARTRATE NANOSPHERES

FIG. 10: AUC SPECTRUM OF ZOLPIDEM TARTRATE NANOSPHERES (BROMOPHENOL BLUE)

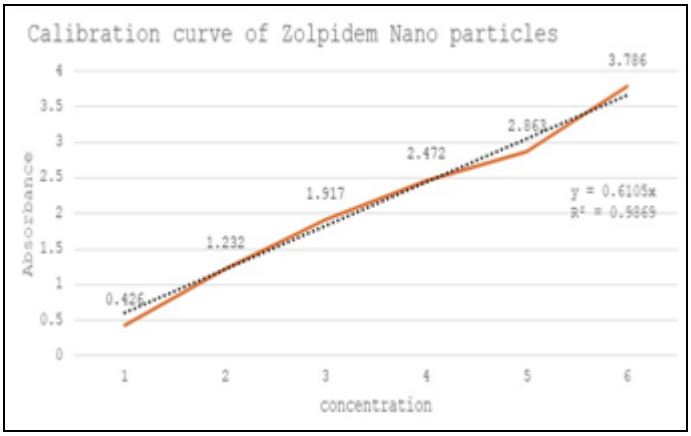

FIG. 11: LINEARITY STUDIES OF ZOLPIDEM TARTRATE NANOSPHERES (BROMO CRESOL GREEN)

Human Plasma Spiking of Zolpidem Tartrate Nanospheres: Zolpidem tartrate nanospheres were spiked into human blood collected in a pre-treated Vacutainer, and the plasma was separated by centrifugation. The separated drug–bound plasma samples were subjected to UV spectral analysis, and linearity studies were carried out. The results were presented in **Table 4** and **Fig. 12-13.**

TABLE 4: LINEARITY VALUES OF THE ZOLPIDEM TARTRATE NANOSPHERES IN THE HUMAN PLASMA SPIKING STUDIES

S. no.	Concentration (ng/mL)	ƛmax	Absorbance
1	100	296	0.997
2	200	296	1.081
3	300	296	1.952
4	400	296	2.995
5	500	296	3.673

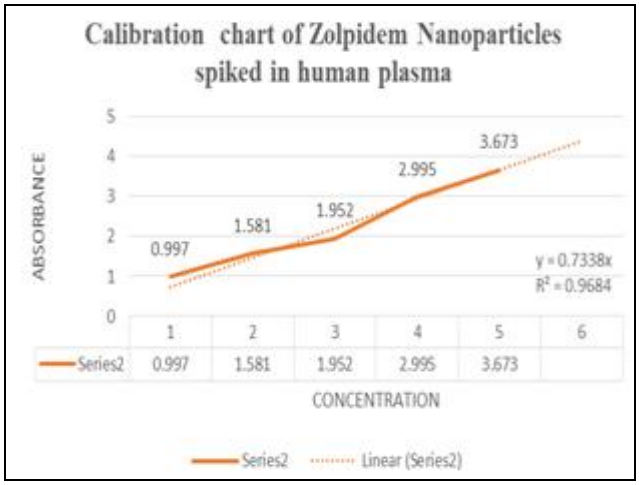

FIG. 12: LINEARITY VALUES OF ZOLPIDEM NANOPARTICLE SPIKED IN HUMAN PLASMA

FIG. 13: UV SPECTRA OF ZOLPIDEM NANOSPHERES SPIKED IN HUMAN PLASMA

Determination of Cmax, Tmax, AUC0–t, and AUC0–α, of Zolpidem Tartrate Nanospheres: Blood samples collected from volunteers were subjected to freeze-thaw cycles and stored at -10º C. Plasma was separated using microcentrifugation technique. Absorbance was measured using UV–Vis spectroscopy. C_{max}, T_{max}, AUC0-t, and AUC0-α values are presented in **Table 6.**

TABLE 6: PHARMACOKINETIC DATA OF THE ZOLPIDEM TARTRATE NANOSPHERES

S. no.	Parameter	Zolpidem Tartrate Nanospheres
1	Cmax (ng/mL)	357.274+/-32.57
2	T_{max} (hr)	0.8333
3	AUC_{0-t}	623.32+/-68.55
4	$AUC_{0-\alpha}$	656.013 +/-358.640

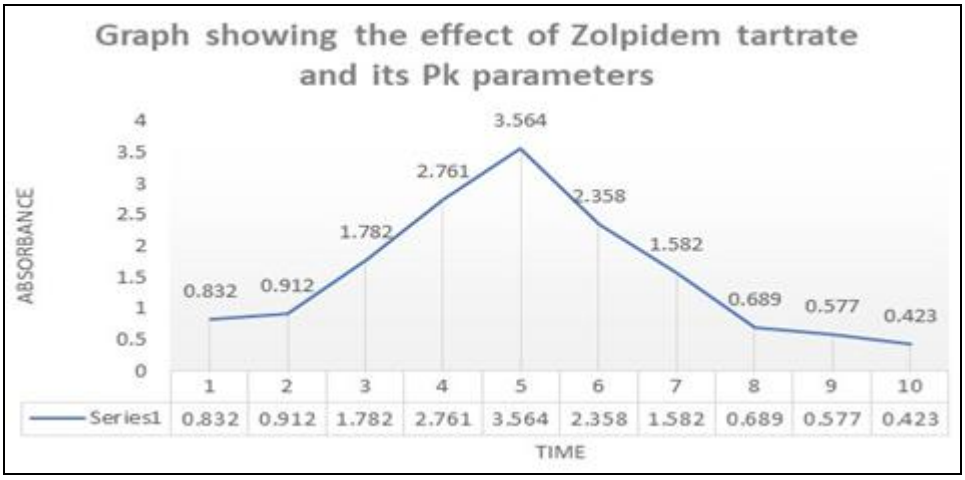

FIG. 14: PK PROPERTIES OF ZOLPIDEM TARTRATE NANOSPHERES

CONCLUSION: In conclusion, a simple, precise, and accurate UV spectrophotometric method was the determination of zolpidem tartrate nanospheres. The nanospheres were accordance with ICH guidelines. Pharmacokinetic parameters, including Cmax, Tmax, AUC0-t, and AUC0-α, were determined for the zolpidem tartrate nanospheres. The results demonstrated reproducibility, with no observed interference from the polymer matrix in the nanosphere formulation.

ACKNOWLEDGEMENTS: The authors express their gratitude to Mr. Ummer Erramangalam, General Secretary; Mr. Kabeer P K, Treasurer; Mr. Mohammed Ashraf, Administrative Officer; and Mrs. Parvathy K T, Superintendent, Karuna College of Pharmacy, for providing the necessary facilities to conduct this research. Appreciation is also extended to Mookambiga Medical College, Kanyakumari District, for their assistance in the pharmacokinetic studies.

CONFLICT OF INTEREST: The authors declare no conflicts of interest regarding this manuscript.

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How to cite this article:
Shanmugakumar SD, Reheeba A, Zeba MA, Mijuna K, Jasmin KS, Sulthana KA, Shruthi K and Rekha SV: Quantitative bioanalytical derivative spectroscopic studies of zolpidem tartrate nanospheres. Int J Pharm Sci & Res 2026; 17(7): 2089-97. doi: 10.13040/IJPSR.0975-8232.17(7).2089-97.