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Spectroscopic determination of PDAC induced coupling reaction and stability indicating stress degradation studies of zolpidem in bulk and pharmaceutical formulation

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ABSTRACT

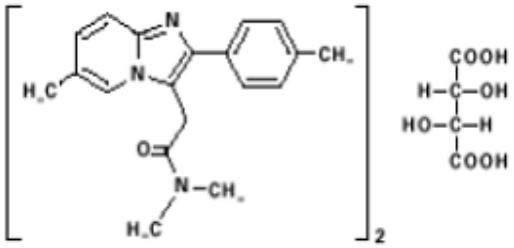
A simple and sensitive spectrophotometric method was developed for the determination of Zolpidem either in API or in pharmaceutical formulations. The method is based on reaction of Zolpidem with paradimethyl aminocinnamaldehyde (PDAC) resulting in the complex formation of orange yellow colored chromogen having absorption maxima at 436 nm. Beer's law is obeyed in the concentration range of 1-5 µg/ml. The proposed method was successfully validated as per the ICH guidelines for API and pharmaceutical preparations of Zolpidem. The percentage recovery studies were performed and the percentage recovery was found to be of 99.38-100.48%, the system was found to be linear and the correlation coefficient (r2) was found to be 0.998, method was found to be precise and the relative standard deviation was found to be 0.511, detection limit was found to 0.3 µg/ml and quantification limit was found to be 1µg/ml respectively. The stress degradation studies were also peformed for the API as per the ICH guidelines, the degradation was observed in oxidative stress and dry heat induced studies.

Keywords: Chromophore, PDAC, Stress degradation, Zolpidem.

INTRODUCTION

Zolpidem tartrate is an imidazopyridine with strong sedative actions [1], minor anxiolytic, muscle relaxant and anticonvulsant properties, it is also a non-benzodiazepine hypnotic used in the treatment of insomnia [2] .Zolpidem is indicated for regulating the sleep by potentiating the actions of inhibitory neurotransmitter γ-aminobutyric acid (GABA) receptors containing α1 subunits in the central nervous system [3]. The chemical name of Zolpidem is N,N,6-trimethyl-2-p-tolylimidazo[1,2-a] pyridine-3-acetamide L-(+)-tartrate (2:1) [4,5].

Zolpidem is official in British, European and Poland pharmacopoeias [5-8]. For the estimation of Zolpidem few analytical methods such as UV, HPLC, HPTLC, Potentiometric methods were reported [9-12]. In the present investigation a spectrophotometric method was developed by coupling Zolpidem in both API and pharmaceutical formuations with PDAC reagent and the method was validated as per ICH guidelines and also performed stability indicating studies for API.



Scheme no.1. Structure of Zolpidem

MATERIALS AND METHODS

The drug sample Zolpidem was obtained as gift sample from Calyx Chemicals & Pharmaceuticals Pvt. Ltd.). All chemicals and reagents were of analytical grade such as PDAC (Himedia RM 152810G), Hydrogen peroxide (SDFCL (SD Fine chem limited), Sodium Hydroxide (Mio chem Pvt Ltd), Hydrochloric acid (Merck Chemicals). Tablet Zolfresh (Abbott group of companies) containing 10 mg of Zolpidem tartrate were procured from local drug store.

Spectroscopic conditions:
ELICO-SL 244 UV/ VIS double beam spectrophotometer, (spectratreats) with PMT detector, was used for the colourimetric estimation of Zolpidem in API and Pharmaceutical preparations.

Reagents:
PDAC (1%):
An accurately weighed quantity of 1g of PDAC reagent was dissolved in 30 ml of methanol and sonicated for 10 min for proper dissolution and the final volume was made to 100ml with methanol.

Hydrochloric acid (1N):
An accurately measured volume of 85 ml of Hydrochloric acid was dissolved in 30 ml of water and the final volume was adjusted to 1000 ml with distilled water.

Hydrogen Peroxide (3%):
An accurately measured volume of 3 ml of Hydrogen Peroxide was dissolved in 30 ml of water and the final volume was adjusted to 100 ml with distilled water.

Sodium Hydroxide (1N):
An accurately weighed quantity of 40 g of Sodium Hydroxide was dissolved in 30 ml of water and sonicated for 10 min for proper dissolution and the final volume was made to 1000ml with distilled water.

Preparation of standard:
Accurately weighed 10 mg quantity of API was dissolved in 100 ml of methanol to give a concentration of 100µg/ml. The final concentration was brought to 10µg/ml by diluting the stock solution with methanol.

Aliquots of standard solutions of Zolpidem were taken into a series of 10 ml volumetric flasks and 1 ml of 1 % PDAC, 2- 3 drops of conc. H_2SO_4 was added and heated for 20 min. The contents in each volumetric flask were finally made upto 10 ml with methanol to get the final concentration ranging from 1µg/ml – 5µg/ml and the absorbance of the orange yellow colored chromogen was measured from 200 – 800nm against the corresponding reagent blank. The amount of Zolpidem was computed from the Beer – Lambert's plot.

Assay of pharmaceutical tablets:
Twenty tablets were weighed and ground to a fine powder using a pestle and a mortar. The average weight of a tablet was calculated. An accurately weighed portion of the powder, equivalent to 100 mg Zolpidem was transferred into a 100 ml volumetric flask. The volume was made up to the mark with methanol, shaken well, and filtered through an ordinary filter paper. The final concentration was diluted to 10 µg/ml with methanol after the addition

1ml of 1 % PDAC, 2- 3 drops of conc. H_2SO_4 and heated for 20 min. The resulting chromogen was measured from 200 – 800nm against the corresponding reagent blank.

Method Validation:
The method was validated for linearity, accuracy, precision, LOD, LOQ as per the ICH guidelines [13, 14].

STRESS DEGRADATION STUDIES:
The stress degradation studies such as hydrolytic (in acidic & alkali medium), photolytic, oxidative and dry heat induced degradations studies were performed for API as per ICH guidelines [13, 14].

1. Hydrolytic degradation under acidic conditions:
Hydrolytic degradation studies was performed by taking 2ml (100µg/ml) of stock solution, to this 1ml of 1N HCL was added and volume was made to 10ml with water, kept at normal conditions for 90min. 5ml of the above solution was pipetted out into 10ml flask and the volume was adjusted with distilled water. Keeping distilled water as a blank, the resulting solution was scanned from 200-400nm.

2. Hydrolytic degradation under alkaline condition:
Hydrolytic degradation studies was performed by taking 2ml (100μg/ml) of stock solution, to this 1ml of 1N NaOH was added and volume was made upto 10ml with water kept at normal conditions for 90min, from this 5ml of solution was pipetted out into 10ml flask and the volume was adjusted with distilled water. Keeping distilled water as a blank, the resulting solution was scanned from 200-400nm.

3. Dry heat induced degradation:
Dry heat induced degradation study was performed by taking drug in petri plate and subjected to a temperature of 70^{o}C for 48 hrs. After 48 hrs 10mg of the drug was taken and diluted with the distilled water such that to get a final concentration of 5µg/ml, Keeping distilled water as a blank , the resulting solution was scanned from 200-400nm.

4. Oxidative degradation:
Oxidative degradation studies was perfomed by taking 1.5 ml (100µg/ml) of stock and to this 1ml of 3 % hydrogen peroxide was added and volume is made up to the mark of 10ml of the flask and kept at room temperature for 15 min. A blank solution was prepared with 1 ml of 3 % w/v hydrogen peroxide into a 10 ml flask and volume is made upto the mark. The resulting solution was stored over night. Both solutions are boiled to remove excess of hydrogen peroxide. Solutions are kept for 15 min and then diluted to a concentration of 5 µg/ml. Keeping distilled water as a blank , the resulting solution was scanned from 200-400nm.

5. Photolytic degradation:
A photolytic degradation study was performed by exposing the sample to near UV light for 30 minutes in a UV chamber. After UV exposure 10 mg of substance was taken and the final dilution was made to get a concentration of 5µg/ml using distilled water. Keeping distilled water as a blank, the resulting solution was scanned from 200-400nm.

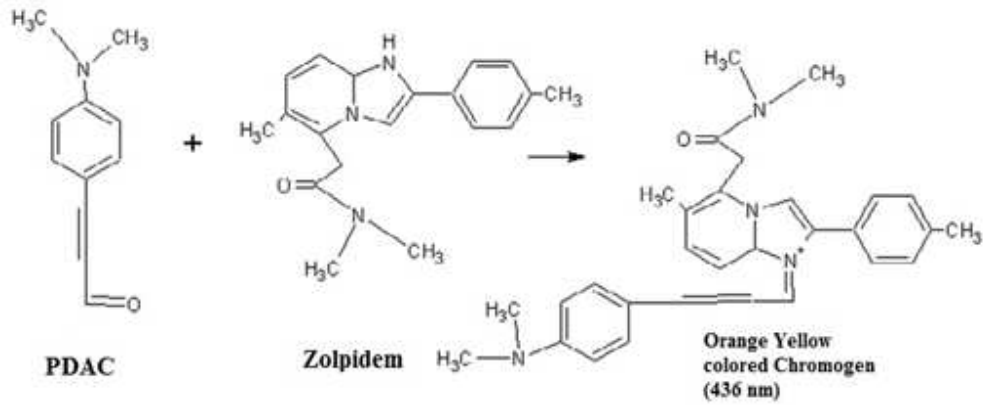
RESULTS AND DISCUSSION

Present investigation is based on new colourimetric method development for the determination of Zolpidem in API and marketed formulation by using PDAC reagent in a concentration of 10 µg/ml solution, an orange yellow colour chromogen was observed. The resulting solution was scaned from 200-800nm. The wavelength maxima of the yellow coloured chromogen showed the maximum absorbance at 436 nm and shown in **scheme. 2** the percentage purity of the given pharmaceutical formulation was found to be 99.17%w/v by the proposed method.

Method validation:
The proposed method was validated for the different parameters such as linearity, precision, accuracy, LOD, LOQ as per the ICH guidelines.

The linearity was observed to obey the Beer's- Lamberts law in concentration ranging from 1-5µg/ml. The linearity plot plotted with concentration against absorbance is showed in **Table 1 and Fig.1.**, correlation coefficient r^2 was found to be 0.998. The Precision studies were performed for six repeated absorbance of same homogenous solution

having the concentration of 1µg/ml and the percentage relative standard deviation was found to be 0.511%, % recovers studies was performed for the 80%, 100% and 120% respectively, percentage recovery of Zolpidem was found to be in between 99.36-100.48 %w/v, detection limit and quantification limit was found to be 0.3µg/ml and 1µg/ml. The result table of the method development and validations was summarized in **Table 2-4.**



Scheme. 2. Showing the reaction of Zolpidem with PDAC

Table 1. Showing the linearity studies for Zolpidem

S.No.	Concentration (µg/ml)	Absorbance at 294 nm
1	0	0
2	1	0.0077
3	2	0.2628
4	3	0.5304
5	4	0.7403
6	5	1.019
	Correlation coefficient (r^2)	**0.9986**

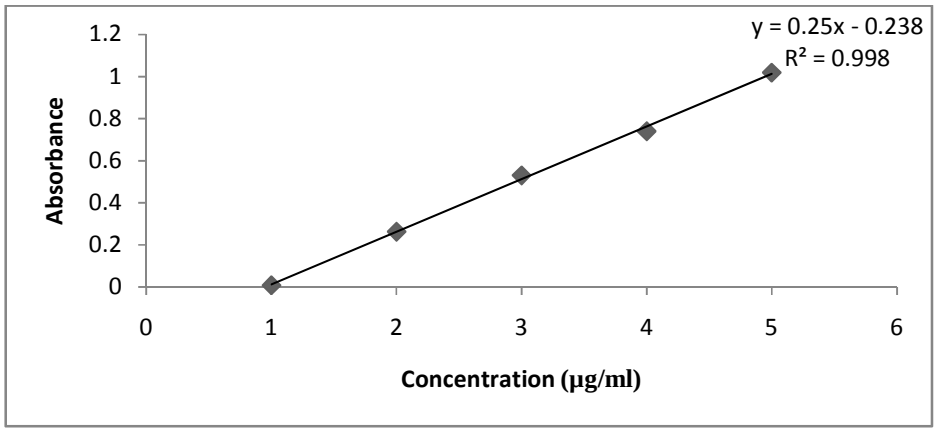


Fig.1. Linearity plot of Zolpidem

Table 2. Showing the precision studies of Zolpidem

S.No	Concentration of the solution(µg/ml)	Absorbance
1	1	0.7624
2	1	0.7661
3	1	0.7643
4	1	0.7632
5	1	0.7652
6	1	0.7543
7	**Mean**	**0.762583**
8	**Standard deviation**	**0.003898**
9	**%RSD**	**0.511**

Table 3. Showing the accuracy studies of Zolpidem

S.No	Accuracy	Amount of Standard added(mg)	Absorbance	Amount recovered (mg)	% Recovery	Mean
1	80%	0.00003	0.0521	0.00988	98.89	99.85
		0.00003	0.0535	0.01014	101.47	
		0.00003	0.0659	0.00992	99.20	
2	100%	0.00003	0.0649	0.00984	98.48	99.39
		0.00003	0.0654	0.00992	99.24	
		0.00003	0.0662	0.0100	100.45	
3	120%	0.00003	0.0802	0.0101	101.37	100.48
		0.00003	0.089	0.0100	100.36	
		0.00003	0.0794	0.0099	99.73	

Table 4. Optical density characterization of Zolpidem

Parameter	Observations
λmax(nm)	294
Complex λmax(nm)	436
Beer's law limits (mg/ml)	1 -5 µg/ml
Molar absorptivity (Liter/mole^{-1}cm^{-1})	252.85
Correlation Coefficient (r^2)	0.998
Regression Equation (Y)*	Y= 0.25x + 0.238
Slope (b)	0.25
Intercept(a)	0.238
%RSD	0.511
LOD	0.3 µg/ml
LOQ	1 µg/ml

Stress degradation Studies:

The stress degradation studies were performed for the Zolpidem (API) as per the ICH guidelines by subjecting the drug to different conditions that stimulate the drug degradation. Further the spectrum of the drug was analysed from 200-400 nm for the degraded changes. The observations are shown in the **Table.5** and spectrum is shown in **Fig.2-6**.

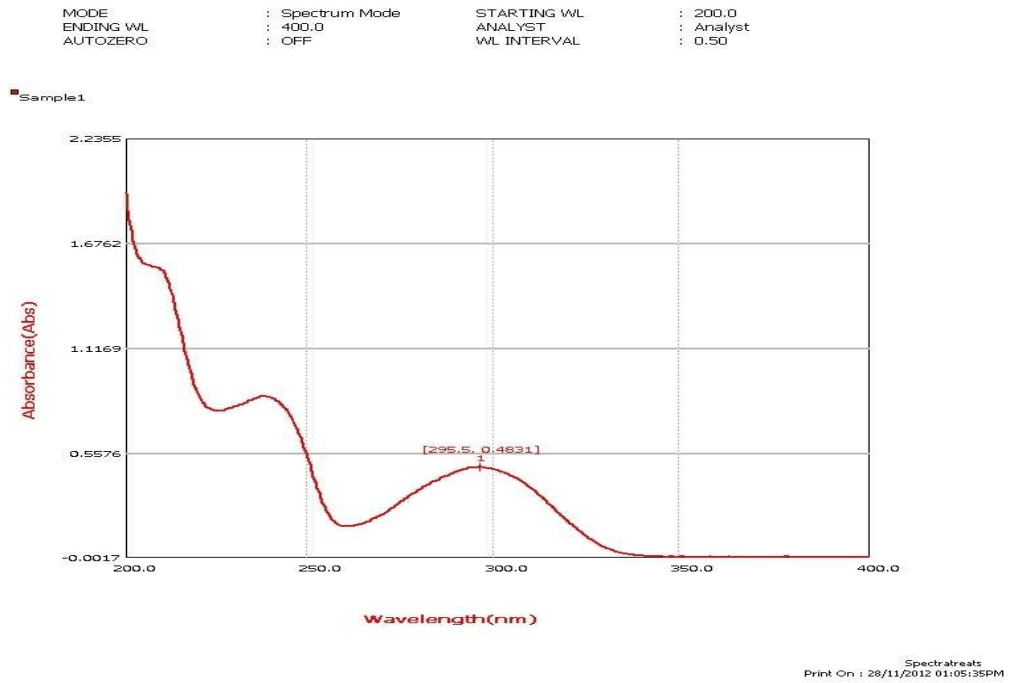


Fig.2. Hydrolytic degradation in acidic conditions.

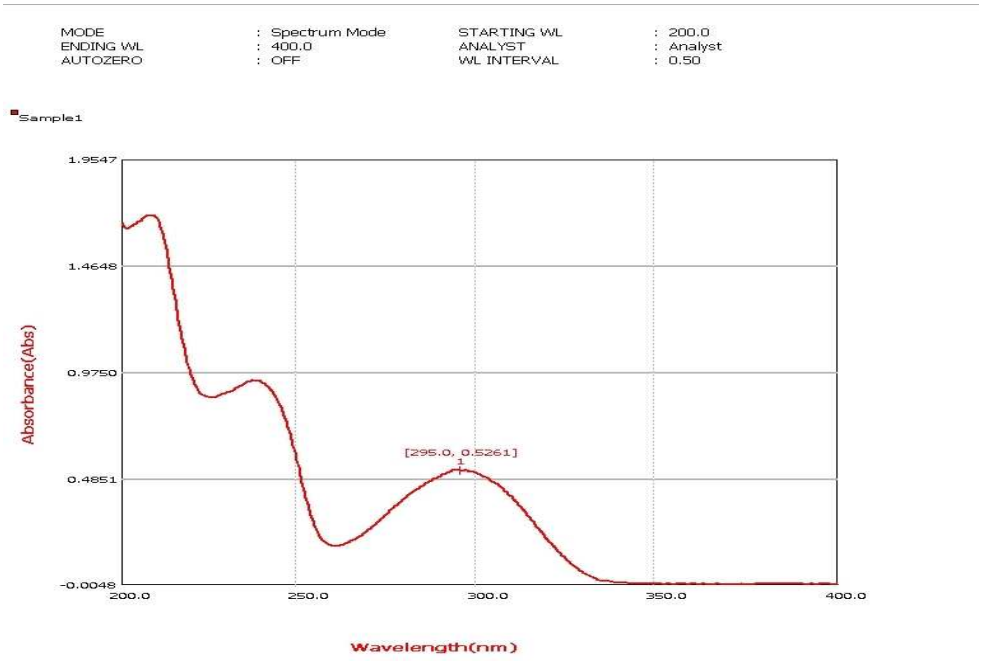


Fig.3. Hydrolytic degradation in alkali conditions.

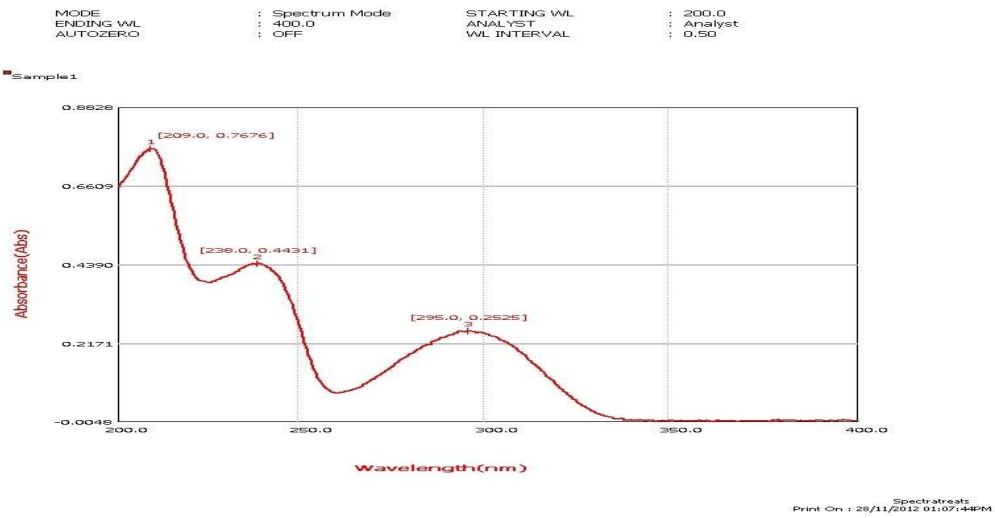


Fig.4. Photolytic degradation studies.

Table 5. Showing the Stress Degradation Studies.

S.No.	STRESS DEGRADATION STUDIES	TIME PERIOD	OBSERVATION
1.	HYDROLYTIC DEGRADATION UNDER ACIDIC CONDITIONS	90 min.	NO DEGRADATION
2.	HYDROLYTIC DEGRADATION UNDER ALKALI CONDITIONS	90 min.	NO DEGRADATION
3.	PHOTOLYTIC DEGRADATION	30 min.	NO DEGRADATION
4.	OXIDATIVE DEGRADATION	15 min.	DEGRADATION WAS OBSERVED
5.	DRY HEAT INDUCED DEGRADATION	48 hrs	DEGRADATION WAS OBSERVED

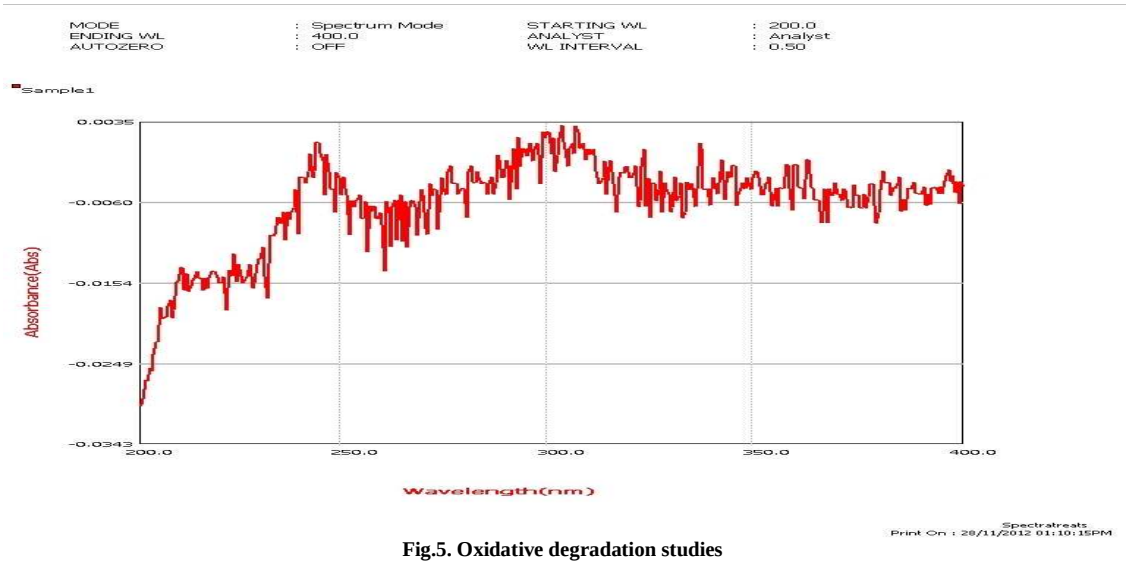


Fig.5. Oxidative degradation studies

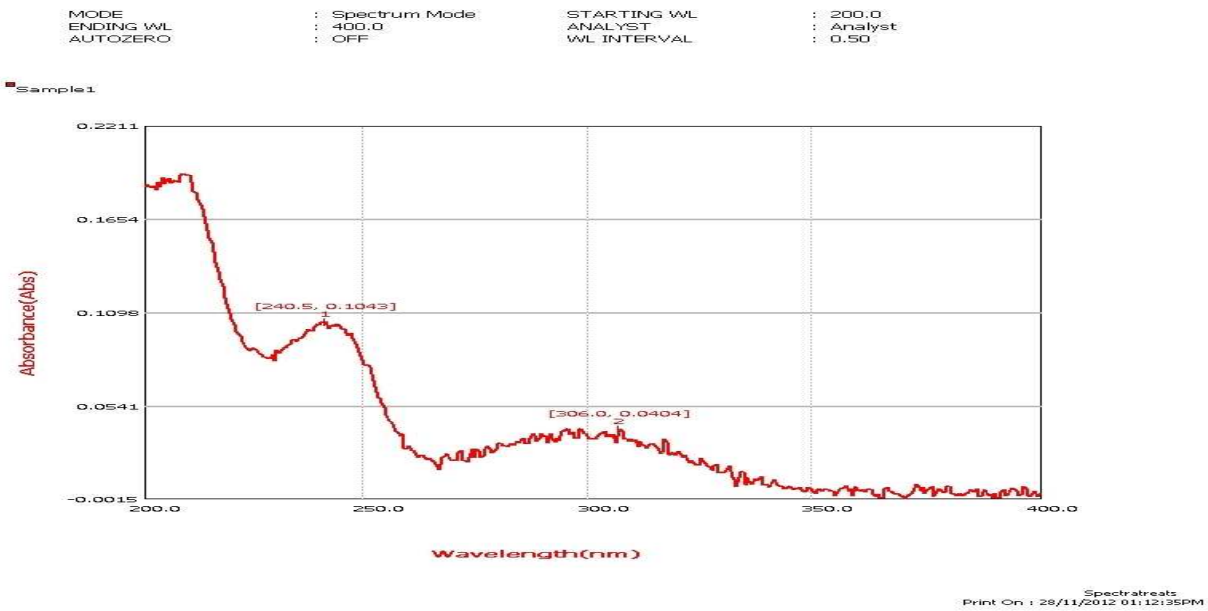


Fig.6. Dry heat induced degradation studies

CONCLUSION

The proposed method for colorimetric estimation of Zolipidem in API and pharmaceutical dosage form was successfully developed & validated as per the ICH guidelines; the results observed for the proposed method were within the standard limit. Stability indicating stress degradative studies were also performed on Zolipidem API as per ICH guidelines. The proposed chromophoric assay method can be used for the routine analysis of Zolpidem in the bulk drug and pharmaceutical dosage form.

REFERENCES

[1] Sweetman SC, Martindale: The Complete Drug Reference, (The Pharmaceutical Press, London, edns 35, **2007**), 933.
[2] O'Neil MJ, (Ed). The Merck Index: An Encyclopedia of Chemicals, Drugs and Biologicals. (Merck & Co., Inc, edn.14, **2006**), 1754.

[3] Bertam GK, Basic and Clinical Pharmacology. Sedative –Hypnotic Drugs. (Mc Graw Hill, edn 14, **2007**), 355.
[4] British Pharmacopoeia, Medicines and Health Care Products Regulatory Agency (MHRA), London, edn 2, **2008**.
[5] United States Pharmacopoeia and National Formulary, Asian Edition, The United States Pharmacopoeia Convention Inc., U.S.A., 2009.
[6] Rajiv Chomwal, Amit Kumar, and Anju Goyal, *J Pharm Bioallied Sci*, **2010,** 2(4), 365–368.
[7] M. P. Mahajan, S. D. Sawant, *International Journal of ChemTech Research*., **2012**, 4, 403-408.
[8] K. Ratna Kumari1, C. Bala Sekaran, *Arch. Appl. Sci. Res*., **2010**, 2 (1), 159-164.
[9] Prakash S. Sarsambi, D. Gowrisankar, *International Journal of ChemTech Research*., **2010**, 2, 1, 282-285.
[10] Bose A, Dash PP, Sahoo MK, *Pharm Methods*., **2012**, 1, 57-60.
[11] Fazel Shamsa and Leyla Amani, *Iranian Journal of Pharmaceutical Research*., **2006**, 1, 31-36.
[12] M. A. Gotardo, L. Pezza, H. R. Pezza, *Ecl. Quím., São Paulo*, **2005**, 30(2), 17-24.
[13] Text on validation of analytical procedures & Methodology ICH Harmonized tripartite guidelines, Q2 (R1), **1994.**
[14] Validation of Analytical Procedures: Methodology (Q2B), ICH Harmonized, Tripartite Guidelines, Geneva, **1996**.