RESEARCH ARTICLE Am. J. PharmTech Res. 2012; 2(6) ISSN: 2249-3387

AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

Stability indicating HPTLC and HPLC determination of Zonisamide in Pharmaceutical Dosage form

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ABSTRACT

Two sensitive and reproducible methods are described for the quantitative determination of zonisamide in the presence of its degradation products. The first method was based on high performance thin layer chromatographic (HPTLC) followed by densiometric measurements of their spots at 238 nm.the separation was on HPTLC aluminium sheets of silica gel 60 F_{254}, using toluene:ethtyl acetate (6:4 v/v), this system was found to give compact spots for zonisamide after development (R_f value 0.49.). The second method based on high performance liquid chromatographic (HPLC) of the drug from its degradation products on reversed phase Vydac column ODS (250 mm × 4.6 mm, 5.0 µ), at ambient temperature using a mobile phase consisting of methanol: water (50:50, v/v) and Retention time was (4.71 min.) Both the methods were validated as ICH guidelines no chromatographic interference from capsule excipients was found. Zonisamide was subjected to acid and alkali hydrolysis, oxidation,and dry heat. The drug was fount to be stable in neutral wet heat and photo- degradation conditions as the proposed analytical methods could effectively separated the drug from its degradation products, they can be employed as stability-indicating.

Keywords: Column liquid chromatography, Thin layer chromatography, Method validation and degradation , Zonisamide

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Received 05 November 2012, Accepted 21 November 2012

INTRODUCTION

Please cite this article in press as Singh R *et al.*, Stability indicating HPTLC and HPLC determination of Zonisamide in Pharmaceutical Dosage form. American Journal of PharmTech Research 2012.

Zonisamide [1,2-benzisoxazole-3-methane sulfonamide] is used as an anticonvulsant in patients with epileptic disorders It has a benzisoxazole structure that is unrelated to other antiseizure drugs. Zonisamide is well absorbed from the gastrointestinal tract T_{max} is 2–6 h following the oral, single dose administration of 200–800 mg in both patients and healthy volunteers Food has no impact on the absorption of zonisamide The therapeutic range of this drug is relatively narrow; high dose often cause side effects such as drowsiness, ataxia, anorexia, decreased spontaneity, mental slowness and weight loss.

Two methods have been reported for the estimation of zonisamide, micellar electro kinetic capillary chromatography and LC method in human plasma. To the best of our knowledge, no stability indicating HPTLC and HPLC methods are reported, for the determination of zonisamide as bulk and in pharmaceutical dosage form. It was felt necessary to develop a stability indicating LC method for the determination of zonisamide as bulk and in pharmaceutical dosage form.[1,2]

MATERIAL AND METHOD:

Materials Required:

Pharmaceutical grade of Zonisamide kindly supplied as a gift sample by Wockhardt pharmaceutical, India. All chemicals and reagents used were of HPLC and analytical grade and were purchased from Merck Chemical. India.

Methods Used:

Instrumentation and chromatographic ConditionsFor HPTLC Densitometry (method 1)

The samples were spotted in the form of bands of width 6 mm with a Camag 100 microlitre sample (Hamilton, Bonaduz, Switzerland) syringe on precoated silica gel aluminium plate 60 F 254, (20 × 10 cm) with 250 µm thickness; (E.Merck, Darmstadt, Germany, supplied by Anchrom Technologists, Mumbai) using a Camag Linomat IV applicator (Switzerland). The plates were prewashed by methanol and activated at 110 ^{o}C for 5 min prior to chromatography. A constant application rate of 0.1 µl/s was employed and space between two bands was 6 mm.The slit dimension was kept at 5 × 0.45 mm and 10 mm/s scanning speed was employed. The monochromator bandwidth was set at 20 nm with K 320 cut off filter, each track was scanned thrice and baseline correction was used. The mobile phase consisted of toluene:ethtyl acetate (6:4 v/v) and 10 mL of mobile phase was used per chromatography. Linear ascending development was carried out in 20 x 10 cm twin trough glass chamber (Camag, Muttenz, Switzerland). (Dimensions: length × width × height = 12 × 4.7 × 12.5 cm). It was saturated (lined on the two bigger sides with filter paper that had been soaked thoroughly with the mobile phase) and the chromatoplate development was carried out in dark with the mobile phase. The optimized chamber saturation time for mobile phase was 30 min at room temperature (25 °C ± 2) at relative humidity of 60% ± 5. The length of chromatogram run was 9 cm and approximately 30 min. subsequent to the development. TLC plates were dried in a current of air with the help of an air dryer in wooden chamber with adequate ventilation. The flow of air in the laboratory was maintained unidirectional (laminar flow, towards exhaust). Densitometric scanning was performed on Camag TLC scanner III in the reflectance-absorbance mode at 238 nm for all measurements and operated by CATS software (V 4.06, Camag). The source of radiation utilized was deuterium lamp emitting a continuous UV spectrum between 190 and 400 nm. [1,2]

For HPLC (method 2)

The HPLC system consists of a pump (model Jasco PU 2080, intelligent HPLC pump) with injecting facility programmed at 20 µL capacity per injection was used. The detector consists of a UV/ VIS (Jasco UV 2075) model operated at a wavelength of 238 nm. The software used was Jasco Borwin version 1.5, LC-Net II/ADC system. The columns used were Vydac ODS (250 mm × 4.6 mm, 5.0 µ), Thermo Hypersil C_{18} (250 mm × 4.6 mm, 5.0 µ) Flexit Pvt Ltd Pune, India. Different mobile phases were tested in order to find the best conditions for separation of zonisamide in presence of its degradation products. The optimal composition of the mobile phase was determined to be methanol: water (50:50 v/v). The flow rate was set to 1.0 mL^{-1} min and UV detection was carried out at 238 nm. The mobile phase and samples were filtered using 0.45 µm membrane filter. All determinations were performed at ambient column temperature. [1,2]

Preparation of Standard and sample solution For HPTLC Densitometry

Stock standard solution was prepared by dissolving 0.10 g of zonisamide in 100 mL methanol 1000 µg mL^{-1} than diluted to get a working stock solution of 100 µg mL^{-1}. The standard solutions were prepared by dilution of the stock solution with methanol to reach a concentration range 200-1400 ng $spot^{-1}$ Two micro liter from each working stock solution was spotted on the TLC plate to obtain final concentration 200-1400 ng $spot^{-1}$.Each concentration was spotted three times on the TLC plate. The plate was developed on previously described mobile phase. The peak areas were plotted against the corresponding concentrations to obtain the calibration graphs.[1,2,3]

Method Validation

Precision of the method was determined with standard drug. System repeatability was determined by three replicate applications and three times measurement of standard solution at the analytical concentration. The repeatability of sample application and measurement of peak area for active compound were expressed in terms of % RSD (relative standard deviation). Method repeatability was obtained from RSD value by injecting three different concentrations on same day for intra day precision. Intermediate precision was assessed by injecting three different concentrations on three different days. The intra day and inter day variation for determination of zonisamide was carried out at three different concentration levels 200, 800, 1200 ng spot $^{-1}$ and 30, 50, 70 µg mL^{-1} for HPTLC and HPLC, respectively. [1,2,3]

Specificity for HPTLC Densitometry

The specificity of the method was ascertained by analyzing standard drug and sample. The spot for zonisamide in sample was confirmed by comparing the R_F and spectra of the spot with that of standard. The peak purity of zonisamide was assessed by comparing the spectra at three different levels i.e., peak start, peak apex and peak end positions of the spot.

For HPLC

The specificity of the HPLC method was determined by the complete separation of zonisamide in presence of its degradation products along with other parameters like retention time (t_R), capacity factor (k), tailing or asymmetry factor (T) etc. [2,3]

Recovery Studies

For both the methods Recovery studies were carried out by applying the method to drug sample to which a known amount of zonisamide corresponding to 80, 100 and 120 % of label claim had been added (standard addition method). At each level of the amount three determinations were performed and the results obtained were compared with expected results.

Analysis of the Marketed Formulation For HPTLC Densitometry

To determine the content of zonisamide in capsule (label claim: 100 mg zonisamide per capsule), the twenty capsules were taken and their contents emptied, weighed and their mean weight determined, then powder equivalent to 100 mg of drug was weighed. Powder was transferred into a 100 mL volumetric flask containing 100 mL methanol and sonicated for 30 min. The resulting solution was centrifuged at 3000 rpm for 5 min. The supernatant was taken and diluted with methanol to get a working stock solution of 100 µg mL^{-1}. And filtered using 0.45-micron filter (Millipore, Milford, MA).different aliquats (2 µL, 8 µL, 12 µL) were spotted to get three different concentrations 200, 800, and 1200 ng spot $^{-1}$ of zonisamide respectively. The plate was developed in the previously described chromatographic conditions. The peak areas of the spots were measured at 238 nm and concentrations in the samples were determined using multilevel calibration curve developed on the same plate under the same conditions using linear regression equation [2]

Accelerated Degradation of Zonisamide

For HPTLC

A stock solution containing 100 mg zonisamide in 100 mL methanol was prepared. This solution was used for forced degradation to provide an indication of the stability indicating property and specificity of proposed method. In all degradation studies the average peak area of standard zonisamide and degradation sample after application 1000 ng spot $^{-1}$ for HPTLC of three replicates were obtained

For HPLC

Preparation of stock solutions: A stock solution containing 100 mg zonisamide in 100 mL methanol was prepared. This solution was used for forced degradation to provide an indication of the stability indicating property and specificity of proposed method. In all degradation studies the average peak area of standard zonisamide and degradation sample after application (50 μg mL^{-1} for HPLC) of three replicates were obtained. [2]

Table 1. Linear regression data for calibration curve

-	HPTLC[a]	HPLC[a]
Linearity range	200-1400ngspot^{-1}	10-80 μg mL^{-1}
r^2	0.9993	0.9988
Slope	114	78377
Intercept [a] **n = 6**	4.8973	41246

Preparation of Acid and Base Induced Degradation products

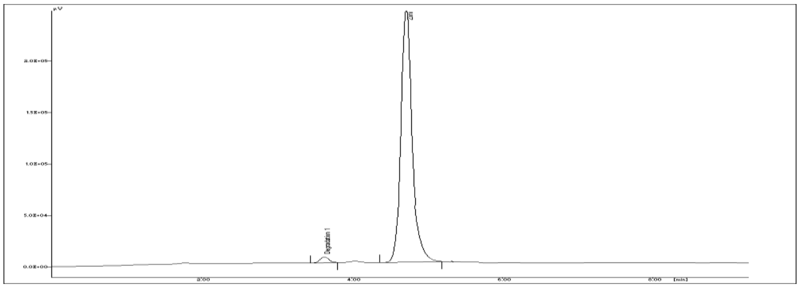

Figure.1 Chromatogram of acidic degradation (6hr at 80^{0}C) of zonisamide; peak 1 degraded (t_R: 3.4 min), peak 2 standard (t_R: 4.5 min).

To 10 mL of stock solution, 10 mL of 5 N HCl and 5 N NaOH were added separately. These mixtures were refluxed for 6 h and 2 h at 80ºC respectively. The forced degradation in acidic and alkaline medium was performed in dark in order to exclude the possible degradative effect of light. (1000 ngspot^{-1}) were applied on a TLC plate and the cromatograms were run as described above (“Method 1”) for the HPLC study the resultant solution was diluted to obtain 50 µg mL^{-1} and 20 µL was injected into the system .[6]

Preparation of Hydrogen peroxide induced Degradation Product

To 10 mL of stock solution, 10 mL of 6% hydrogen peroxide was added separately. The solutions were heated in boiling water bath for 10 min to remove completely the excess of hydrogen peroxide and then refluxed for 2 h at 80^{o}C. (1000 ngspot^{-1}) were applied on a TLC plate and the cromatograms were run as described above (Method 1) for the HPLC study the resultant solution was diluted to obtain 50 µg mL^{-1} and 20 µL was injected into the system as described in (Method 2) [6]

Dry heat Degradation Product

The standard drug was placed in oven at 80ºC for 8 h to study dry heat degradation.(1000 ngspot^{-1}) were applied on a TLC plate and the chromatograms were run as described above (Method 1) for the HPLC study the resultant solution was diluted to obtain 50 µg mL^{-1} and 20 µL was injected into the system as described in (Method 2) [8]

Photochemical Degradation Product

The photochemical stability of the drug was studied by, exposing the stock solution (1 mg mL^{1}) to direct sunlight for 12 h on a wooden plank. (1000 ngspot^{-1}) were applied on a TLC plate and the cromatograms were run as described above (Method 1) for the HPLC study the resultant solution was diluted to obtain 50 µg mL^{-1} and 20 µL was injected into the system . Fig.No.2 [10]

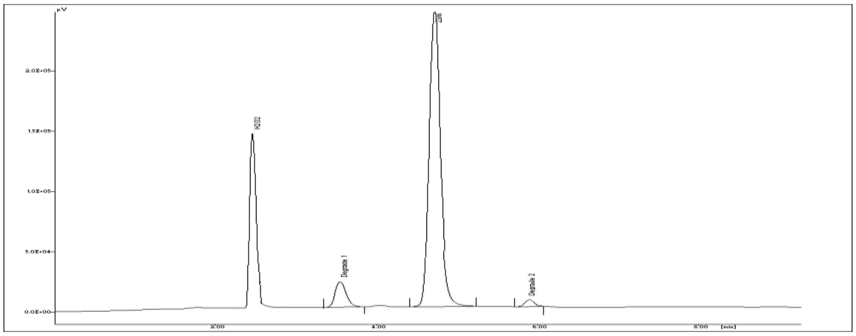

Figure. 2. Photochemical degradation (2hr at 80^{0}C) of zonisamide; peak 1 degraded (t_R: 3.7 min), peak 2 standard t_R: 4.5 min).

Neutral Hydrolysis

Stress testing under neutral conditions was studied by refluxing the drug in water for 12 h at a temperature of 80ºC. (1000 ngspot^{-1}) were applied on a TLC plate and the cromatograms were run as described above (Method 1) for the HPLC study the resultant solution was diluted to obtain 50 µg mL^{-1} and 20 µL was injected into the system . [11]

RESULT AND DISCUSSION

Optimization of procedure For HPTLC Densitometry

Initially two different combinations of mobile phases were tried for the method development. Hexane: ethyl acetate and Toluene: Ethyl acetate in different proportions were tried. It was found that in the Hexane: ethyl acetate the movement of the drug was very less and poor resolution was observed. In Toluene: ethyl acetate (6:4, v/v) the R_F of zonisamide was 0.49 with compact spot and good peak shape, also all the components were properly resolved when scanned densitometrically at 238 nm. The R_F of all the components were between 0.2 to 0.6. Therefore this mobile phase combination was choosen for the validation studies. The saturation time for mobile phase was 30 min at room temperature (25^{o}C ± 2) at relative humidity of 60 % ± 5.

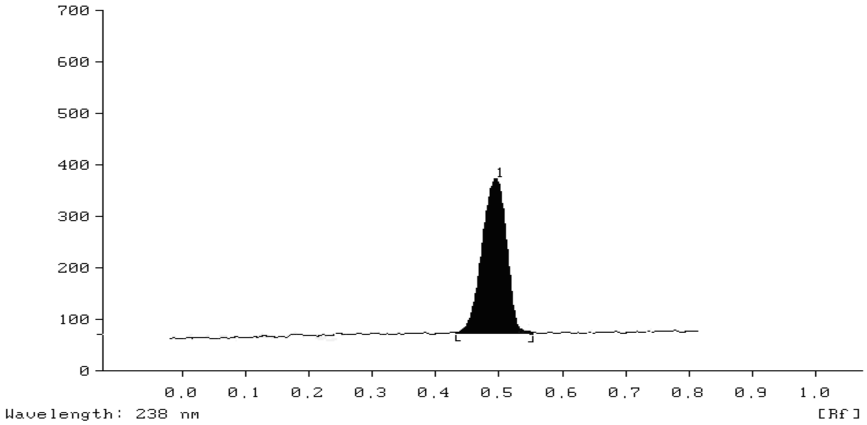

Figure.3 . Densitogram of standard drug (500 ng spot $^{-1}$) R_f : 0.49 measured at 238 nm, mobile phase, Toluene: ethyl acetate (6:4, v/v)

Linearity

Calibration graph was found to be linear, that is adherence of the system to Beer s law was found over the concentration range 200-1400 ng spot $^{-1}$ (r^2 = 0.9993).and 10-80 µg mL^{-1} (r^2 = 0.9988). Linearity was evaluated by determining six standard working solutions containing

Precision

For HPTLC Densitometry

The repeatability of sample application and measurement of peak area were expressed as % RSD and was found to be 1.28 and 1.58.The % RSD for within-day and day to day analysis found to be < 2%.

For HPLC

for within run Precision and between run Precision of the proposed HPLC method were determine by assays the capsule six time per day for conservative 6 days and expressed as %

RSD the % RSD for intra-day and inter day precision was found to be 1.25 and 1.32 for HPLC

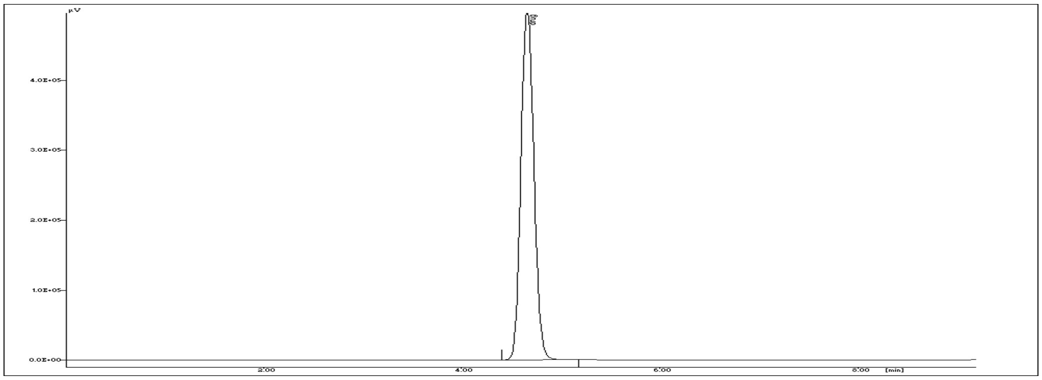

Figure.4. Chromatogram of Zonisamide (20 µg mL^{-1}), t_R : 4.71

Table .2 The standard deviation of peak areas was calculated for each parameter and % RSD was found to less than 2%.

S.N.	Parameter	SD	%RSD
1.	Mobile phase composition (± 0.1 ml)	1.52	1.36
2.	Amount of mobile phase (±5 %)	1.84	1.01
3.	Time from spotting to chromatography (±20 min)	0.79	0.27
4	Time from chromatography to scanning (±5 min)	0.91	0.57

For HPLC

Each factor selected (except columns from different manufacturers) to examine were changed at three levels. One factor at a time was changed to estimate the effect. Thus, replicated injections (n = 3) of standard solution at three different concentration levels were performed. The results show that the results remained unaffected by small variation of these parameters.

Robustness testing for HPTLC densitometry .

Table 3 Robustness testing for HPTLC densitometry

Factor	Level	Chromatographic changes	
		t_R	T_e
A: Flow rate (mL^{-1}min)			
0.9	-1	4.79	1.12
1	0	4.70	1.15
1.1	+1	4.58	1.18
Mean ± SD (n=6)		4.69 ± 0.06	1.15 ± 0.03
B. % of methanol in the mobile phase (v/v).			
48	-2	4.52	1.24
50	0	4.72	1.16
52	+2	4.82	1.19
Mean ± SD (n=6)		4.68 ± 0.13	1.19 ± 0.05
C. Columns from different manufacturers			
Thermo		4.70	1.22
Vydac		4.74	1.15
Mean ± SD (n=6)		4.72 ± 0.03	1.18 ± 0.04

Summary of Validation Parameters by HPTLC and HPLC Methods

Table 4 Validation Parameters by HPTLC and HPLC Methods

Parameter	HPTLC	HPLC
Linearity range	200-1400 ng spot-1	10-80 µg mL-1
Correlation coefficient	0.99930	0.9988
Limit of detection	0.20 µg mL-1	50 ng spot-1
Limit of quantitation	0. 50 µg mL-1	80 ng spot-1
Recovery (n=6)	99.68	99.63
Repeatability % RSD (n=3)	1.22	0.58
Inter-day % RSD (n=3)	0.79	1.54
Robustness	Robust	Robust
Specificity	Specific	Specific

Chromatographically analysis of pharmaceutical formulation.

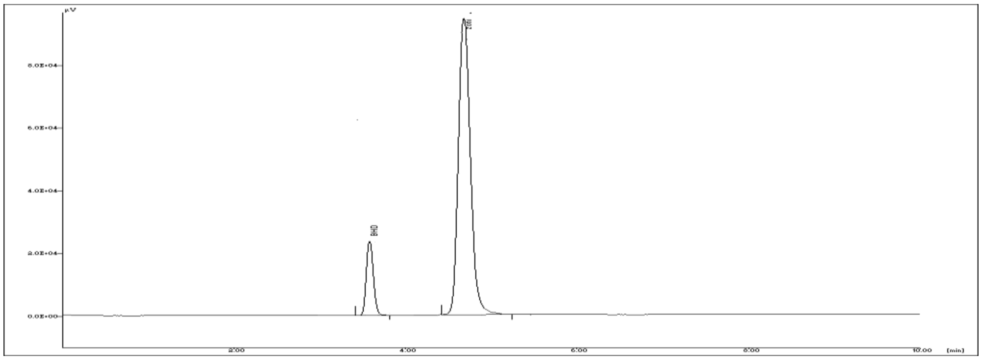

Figure. 5. Chromatogram of alkaline degraded (2hr at 80^0C) of zonisamide ; peak 1 degraded (t_R: 3.7 min), peak 2 standard t_R: 4.5 min).

Specificity For HPTLC Densitometry

The peak purity of zonisamide was assessed by comparing their respective spectra at peak start, peak apex and peak end position of the spot assessed the peak purity of zonisamide.

Stability in Sample Solution For HPTLC Densitometry

Solution of three concentrations 400, 800, and 1200 ng spot^{-1} for zonisamide were prepared from sample solution and stored at room temperature for 24 h applied on HPTLC plate, and after development of the densitogram there was no additional peak.

For HPLC

Three different concentrations of zonisamide 30, 50, and 70 µg mL^{-1} were prepared from sample solution and stored at room temperature for 3 days. No additional peak was found in the chromatogram that means the drug is stable in sample solutions.

Analysis of the marketed formulation

The spots at R_F 0.49 ± 0.03 was observed in the densitogram of the drug. Samples extracted from capsule experimental results of the amount of zonisamide capsule, expressed as percentage of label claim were in good agreement with the label claims there by suggesting that there is no interference from any excipients. The drug content was found to be 99.61%±1.75 (%RSD of 1.76)

Stability indicating property of Zonisamide

Zonisamide were found to be susceptible to all hydrolytic conditions where both the drugs were found to be degraded to about 56-80%, subsequently the studies were done at lower temperatures viz, 60°C and room temperature for alkaline and acidic hyrolysis. On Thermal, UV and Humidity (77%RH) stress where both the drugs showed a degradation of about 18-24% on exposure for a period of 7 days. The degradation products and drugs carry the numerical notations in accordance with the sequence in which the peaks appeared from left to right on the HPTLC chromatogram.

For HPTLC

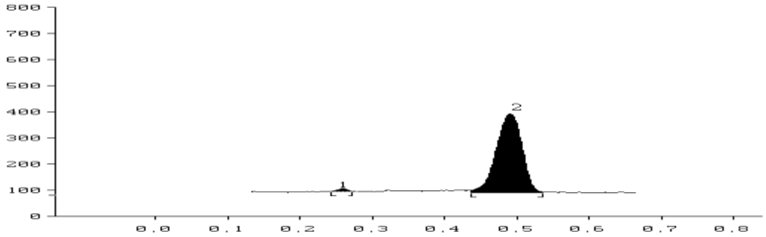

Figure. 6 Densitogram of of acidic degradation (6hr at 80^{0}C) of zonisamide; peak 1 degraded (R_f : 0.28), and peak 2 standard (R_f: 0.49).

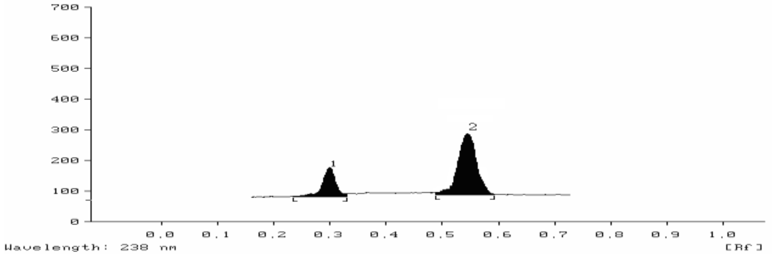

Figure 7 Densitogram of alkaline degraded (2hr at 80^{0}C) of zonisamide, peak 1 degraded (R_f:0. 29), and peak 2 standard (R_f: 0. 51).

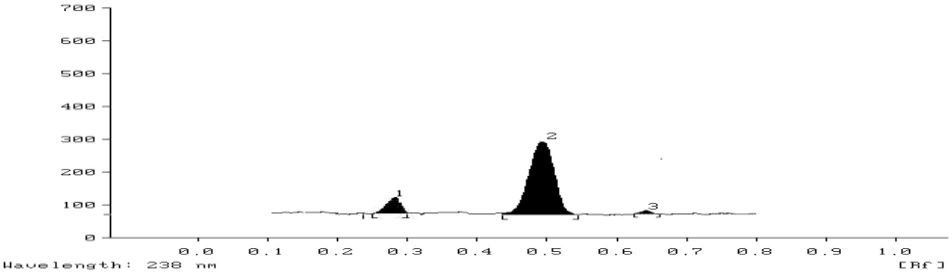

Figure 8. Densitogram of H_2O_2 (6.0%, refluxed for 2.0h, at 80ºC) zonisamide peak 1 degraded(R_f : 0.27), and peak 2 standard (R_f : 0.48), peak 3 degraded (R_f : 0.65).

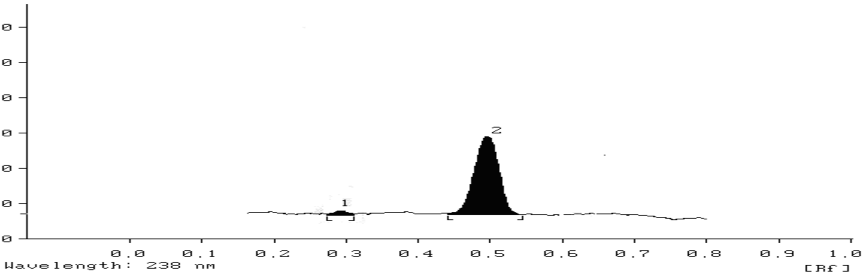

Figure. 9. Densitogram of dry heat (2 hr at 80^{0}C) of zonisamide, peak 1 degraded (R_f: 0.3), and peak 2 standard (R_f: 0.46)

For HPLC

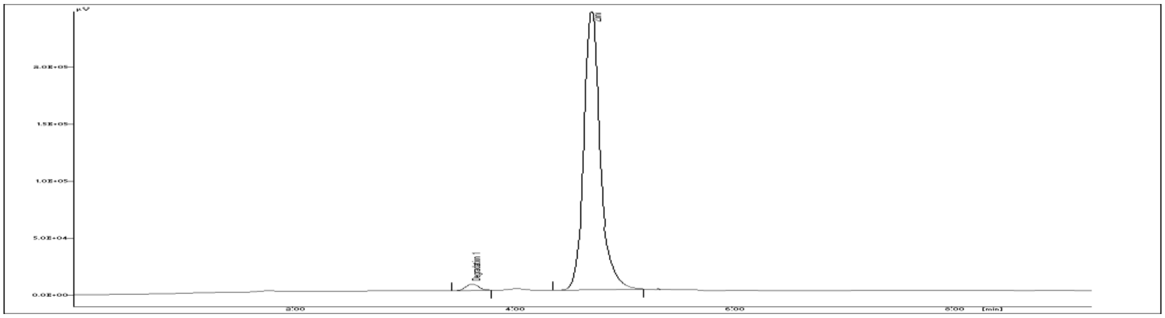

Figure.10 Chromatogram of acidic degradation (6hr at 80^{0}C) of zonisamide; peak 1 degraded (t_R: 3.4 min), peak 2 standard (t_R: 4.5 min).

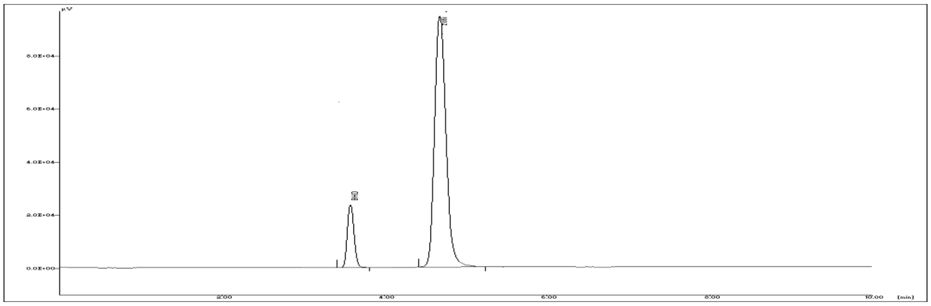

Figure. 11, Chromatogram of alkaline degraded (2hr at 80^{0}C) of zonisamide; peak 1 degraded (t_R: 3.7 min), peak 2 standard t_R: 4.5 min).

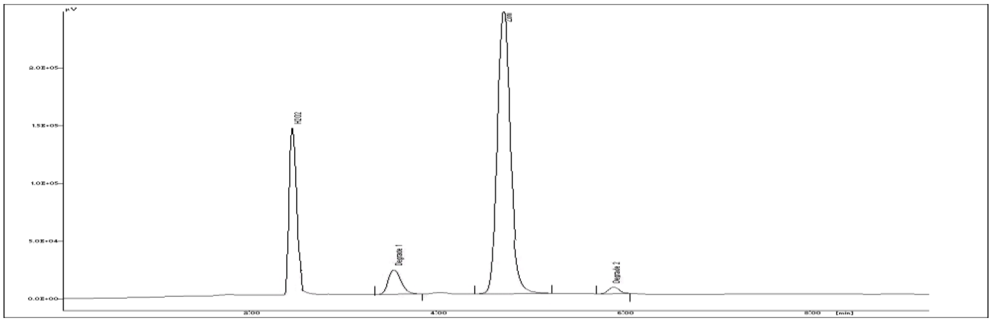

Figure 12, Chromatogram of H_2O_2 (6.0%, refluxed for 2.0h, at 80°C) zonisamide; peak 1 degraded (t_R: 2.7 min), Peak 2 standard t_R: 4.51 min), peak 3 degraded (t_R: 5.8 min).

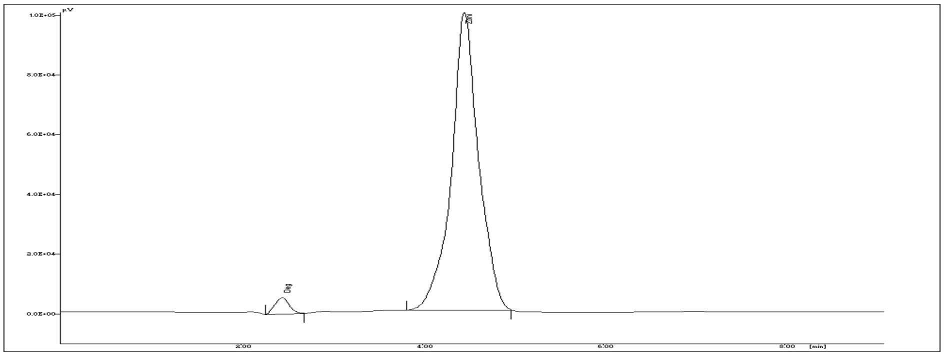

Figure 13.Chromatogram of dry heat (2 hr at 80^0C) of zonisamide; peak 1 degraded t_R: 2.61 min), peak 2 standard (t_R: 4.1 min).

Method validation

The recovery range (accuracy) for Zonisamide were found to be in the range 98.42 to 101.69% and 98.27 to 100.04. The relative standard deviation ranged from 0.77 to 1.60%. Precision was ascertained by replicate analysis of homogenous samples of tablet powder. Assay precision was expressed as the relative standard deviation (RSD, %), found to be ≤ 2.0% for both the drugs. Intra-day precision was determined and %RSD was found to be within limits; inter-day precision were determined by replicate analysis of the solutions on different days. The %RSD was found to be more, as the solution analysed on 11th day showed the presence of additional peaks at tr_3.11 and 4.50 (Peak **5** for DIAC and Peak **7** for ACEL as they appear in chromatogram, Figure 14).

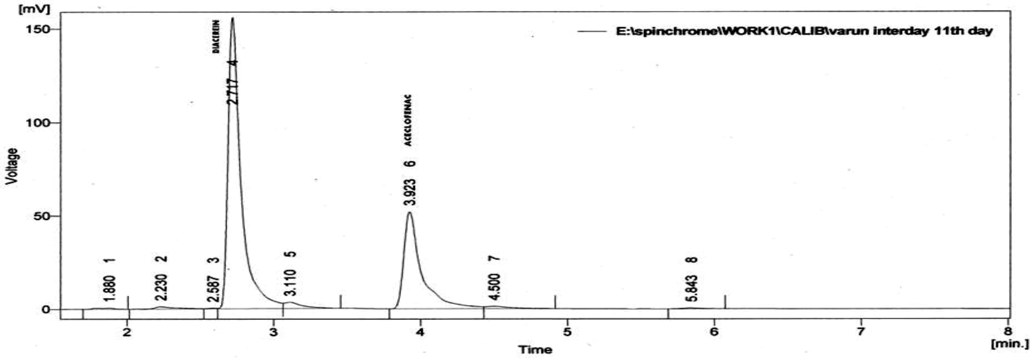

Figure 14: Chromatogram of sample under Inter day precision study (11th day)

LOD and LOQ For HPTLC Densitometry

Signal to noise ratios 3:1 and 10:1 were considered as LOD and LOQ respectively. The LOD and LOQ were found to be 50 and 80 ng $spot^{-1}$

CONCLUSION

The proposed HPTLC and HPLC methods provide simple, accurate, reproducible and stability indicating for quantitative analysis for determination of zonisamide in pharmaceutical dosage form, without any interference from the excipients and in the presence of its acidic, alkaline, oxidative and photolytic degradation products. Both the chromatographic methods were validated as per ICH guidelines. Statistical tests indicate that the proposed HPLC and HPTLC methods reduce the duration of analysis and appear to be equally suitable for routine determination of zonisamide in pharmaceutical dosage form in quality control laboratories, where economy and time are essential. This study is a typical example of development of a stability indicating assay, it is one of the rare studies where forced decomposition was done under all different suggested conditions and the degradation products were resolved. Hence it is proposed for the analysis of the drug and degradation products in stability samples in industry. The method however, is not suggested to establish material balance between the extent of drug decomposed and formation of degradation products. As the method separates the drug from its degradation products, it can be employed as a stability indicating one.

ACKNOWLEDGEMENT:

The authors are thankful to Department of Pharmaceutics, Bharati Vidyapeeth Deemed University, Poona College of Pharmacy, Erandwane, Pune-411 038, for providing the necessary facilities to done this work.

REFERENCES:-

1. Centar for Drug Evaluation and Research (CDER), Reviewer Guidance of Validation of Chromatographic Methods, November 1994.
2. Juenke J, Brown PI, Urry FM, McMillin GA. Drug monitoring and toxicology: a procedure for the monitoring of levetiracetam and zonisamide by HPLC- UV. J. Anal. Toxicol., ISSN, 0146-4760.
3. Fried, J.Sharma In; Thin Layer Chromatography, Techniques and applications, 3rd ed^{n}, Marcel dekker, NewYork, 1994.
4. ICH, Q2 (R1), Harmonized Tripartite Guideline, Validation of analytical procedures: Text and methodology, IFPMA, in: Proceedings of the international conference on harmonization, Geneva, November 2005.
5. ICH, Q1A (R2), Harmonized Tripartite Guideline, stability testing of new drug Substances and Products, in: Proceedings of the international conference on harmonization, Geneva February 2003.
6. Singh.S, Bakshi. M; Guidance on Conduct of: Stress Tests to Determine Inherent stability of drugs, Pharmaceutical on Line, April 2000, 1-14.

7. ICH, Q1B, Harmonized Tripartite Guideline, stability testing: Photostability testing of new drug substances and products, In: Proceedings of the international Conference on Harmonization, Geneva November 1996.
8. Wilensky AJ, Friel PN, Ojemann LM, Dodrill CB, McCormick KB, Levy RH1985 Epilepsia .
9. Masuda Y, Utsui Y, Karasawa T, Yosh-ida K,Shimizu M 1979 Epilepsia 20:623–633.
10. 10. Sackellares JC, Donofrio PD, Wagner JG,Abou-Khalil B, Berent S, Aasved-Hoyt K (1985) Epilepsia 26:206–211
11. Taylor CP, MacLean JR, Bockbrader N1986 In: Meldrum BS, Porter RJ (eds) New anticonvulsant drugs. John Libbery & Company Ltd, London
12. 12. K.H. Liu, Y.K. Lee, Y.E. Sunwoo, K.S. Yu, W. Kang, et al. High-Performance Liquid Chromatographic Assay of zonisamide (1, 2-benzisoxazole-3-methanesulfonamide) in human plasma using a solid phase extraction technique. J. Chromatographia Aprial 2004, 59, (no.7/8).
13. Furuno K, oishi R, Gomita Y, Eto K. A high-performance liquid chromatography method for determination of zonisamide in Human serum by Solid-Phase Extraction technique. J. Chromatogr B Biomed Appl. 1994 ,17; 656(2):456-9.s
14. Yoshida T, Imai K, Motohashi S, Hamano S, Sato M.Simultaneous determination of zonisamide, carbamazepine and carbamazepine-10,11-epoxide in infant serum by high-performance liquid chromatography. J. Pharma. Biomed. Anal., ISSN 0731-7085.