Original Research

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF ZONISAMIDE IN PURE AND PHARMACEUTICAL DOSAGEFORM AND COMPARATIVE STUDY OF MARKETED PRODUCT BY USING RP-HPLC METHOD

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ABSTRACT

A simple and reproducible method was developed for Zonisamide by Reverse Phase High Performance Liquid Chromatography (RP-HPLC). Zonisamide was separated on C18 column [4.6x150mm, particle size 3µm], using the UV detection of 242nm. Isocratic elution of methanol (MeOH) was used as a mobile phase with the flow rate of 1.5(mL/min) and run time 6 minute, eventually 85:15 v/v MeOH and water was being set with the flow rate of 1.5mL/min. The retention time of Zonisamide was 2.047 min. The statistical validation parameters such as linearity, accuracy, precision, inter-day and intra-day variation were checked, further the limit of detection and limit of quantification of Zonisamide concentrations were found to be 0.02012µg/mL and 0.06098µg/mL. Recovery studies of Zonisamide were within 94.20%to 99.40% of indicating that the proposed method can be adoptable for quality control analysis of Zonisamide.

KEY WORDS: RP-HPLC, UV spectrophotometry, Methanol, Zonisamide.

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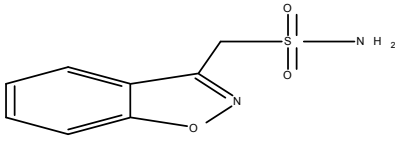
1.INTRODUCTION

Zonisamide is a [1-(1, 2-Benzoxazol-3-yl) methane sulphonamide]benzisoxazole deriva-vative, used as an adjunctive antiepileptic in the treatment of partial seizure [1-3]. Zonisamide may be a carbonic-anhydrase [4-5] inhibitor although this is not one of the primary mechanisms of action. Zonisamide may act by blocking repetitive firing of voltage-gated sodium channels leading to a reduction of T-type calcium channel currents or by binding allosterically to GABA receptor [6].Various UV spectrophotometric methods for estimation of Zonisamide based on condensation [7], complex and redox reaction were developed. Several methods have been reported for analysis of Zonisamide using gas chromatography (GC) [8], micellar electrokinetic [9-10]capillary chromatography[11],enzyme immunoassay, high performance liquid chromatography (HPLC) with UV detection using solid phase extraction . HPLC methods for determination of degradation products for Zonisamide were also reported [12]. Ion pair HPLC[13], RP-HPLC, stability indicating HPLC, LC method and HPTLC method for simultaneous determination of Lamotrigine, Zonisamide and Levetiracetam in human plasma were also developed[14]. So, here we have developed simple, economic, precise & accurate method for the quantitative estimation of Zonisamide in bulk drug and its pharmaceutical dosage form.

Chemical structure of Zonisamide:



2. MATERIALS AND METHODS

2.1.Chemicals and Reagents: HPLC grade Methanol and HPLC grade Water procured from E.Merck (Ahmedabad).All reagents and chemicals were used of Analytical Grade. Gift sample of Zonisamide was supplied by Sun Pharma Drugs Private Ltd. Plot no. 754, Setipool, P.O- Ranipool, East Sikkim-737135.Marketed formulation was procured from local market.

2.2. HPLC Instrumentation: The liquid chromatographic system consisted of following components: Aglient HPLC model 1120 containing LC-Isocratic Pump, variable wavelength prominence UV /VIS detector, Hamilton syringe (50µL) and an injector with a 20µl loop. Chromatographic analysis was performed using Agilent EZChrom Elite software (with Dongle) Agilent C18- 4.6 x 150mm, 3µm particle size.

2.3.Preparation of standard stock solution: Accurately weighed 100 mg of Zonisamide was transferred into 100 ml volumetric flask and dissolved in methanol and diluted up to the mark with methanol(1000ppm).Then taken 5ml solution transferred into 50 ml volumetric flask form 1000ppm solution and volume diluted up to the mark with mobile phase(100ppm).

2.4. Chromatographic Conditions: Solvent MeOH and solution were filtered through 0.45µm membrane filter. The measurements were carriedoutwith an injection volume of

20 μL, flowratewas set to 1.5 mL/min, and UV detection was carried out at 242 nm. All

determinations were done at ambient column temperature (20^0C). The chromatograms of the prepared standard solution of Zonisamide were

recorded under the above optimized chromatographic conditions.

2.5. Test Preparation: Ten capsule of Zonisep and ten capsules of Zonimid separately were weighed, their mean weight, net content in case of capsule determined and crushed in mortar. An amount of powdered mass equivalent to 50 mg of Zonisep and 50mg of Zonimid were transferred into a 100 ml volumetric flask separately containing 20 ml methanol, shaken for 5min and then diluted to volume with methanol and filtered and this solution volume makeup with methanol in 100ml volumetric flask(1000ppm). Then taken 1ml solution transferred into 10 ml volumetric flask form 1000ppm solution and volume diluted up to the mark with mobile phase(100ppm).

3. RESULTS AND DISCUSSION

3.1. Method development. Methanol is a polar solvent being widely used in RP-HPLC. The primary effects it was found that the peak area was increases with increases in the concentration of sample. The chromatogram revealed that with increased in methanol concentration the retention time was reduced. The peak obtained was best acceptable at the pure methanol concentration. The peak was found sharp and reproducible. To determine the effect of flow rate, the programmed controller was set at different flow rates 1mL/min, 1.5mL/min, operate were performed at each flow rate. The peak obtained was broad and showed severe tailing in 1mL/min flow rate. The optimum flow rate was also chosen keeping in mind the recommended flow rate for a column with a given internal diameter.

3.2. Method Validation. The developed analytical method was subjected to validation with respect to various parameters such as linearity, limit of quantification (LOQ), limit of detection (LOD), accuracy, precision, recovery studies, specificity and robustness as per the ICH guidelines [15-16].

3.2.1. Specificity. The specificity of the method was evaluated by assessing interference from exipients in the pharmaceutical dosage form prepared as a placebo solution. The specificity of the method for the drug was also established by checking for interference with drug quantification from degradation products formed during the forced degradation study. The peak purity of the Zonisamide was found satisfactory under different stress conditions. There was no interference of any peak of degradation product with drug peak.

3.2.2. Linearity and Range. For linearity, six-point calibration curve was obtained in a concentration range from 10–60 μg/mL for Zonisamide.The response of the drug was found to be linear in the investigation concentration range, and the linear regression equation for was y = 411016x-1E+06 with correlation coefficient 0.999 where x is the concentration in (μg/mL) and y is the peak area in absorbance unit.

3.2.3. Precision. The precision of the proposed method was ascertained by actual determination of three replicates of a fixed concentration of the drug (10µg/ml) within the Beer's range and finding out the average peak area by the proposed method. The %RSD for Intraday and Inter day precision were found to be 0.0001 and 0.08 respectively. Low values of %RSD indicate that the proposed method is accurate. The data is shown in Table (3, 4).

3.2.4. Accuracy (% Assay). Accuracy study was assessed by determination of the % assay of Zonisep and Zonimid in market formulation. The % recovery of Zonisep and Zonimid were 98.66%, 98.44% respectively, that was satisfactory Table (8).

3.2.5. Recovery Studies. For the evaluation of accuracy of the method, the drug was spiked at three different concentrations (20, 40, 30, and 60 µg/mL) in a mixture of stressed sample. The area obtained was used to calculate the recovery of the added drug. The mean recovery of Zonisamide was between 94.20% and 99.40%, which was satisfactory.

Table 1: HPLC Statistical Data of Zonisamide.

Parameter	RP-HPLC
Analytical wavelength	242
Range	10-60ppm
Slope	41101
Intercept	1E+06
Regression coefficient	0.999
Flow rate (ml/min)	1.5
Mobile phase	methanol
Retention time	2.048
LOD (µg/ml)	0.02012
LQD (µg/ml)	0.06098

Table 2: Calibration curve of Zonisamide by HPLC

Concentration (µg/ml)	Peak Area at 242nm
10	3151786
20	6917029
30	10675442
40	15200479
50	19341504
60	23563188

3.2.6. Limit of Detection (LOD)/Limit of Quantitation (LOQ). The LOD was determined on the basis of signal to noise ratios and was determined using analytical response of three times the background noise. LOQ was determined as the lowest amount of analyte that was reproducibly quantified above the baseline noise following triplicate injections. Both LOQ and LOD were calculated on the peak area using the following equations:

LOQ = $10\times N/B$ and LOD = $3\times N/B$

Where N is the standard deviation (SD) of the peak areas (triplicate injections) of the drug and B is the slope of the corresponding calibration curve. The limit of detection and limit of quantification were evaluated to obtain signal to noise ratio of 3: 1 for LOD and 10: 1 for LOQ. The LOD and LQD value were found to be 0.02012(μg/mL) and 0.06098(μg/mL).

3.2.7. Robustness. The robustness was studied by evaluating the effect of small but deliberate variations in the chromatographic conditions. The conditions studied were flow rate (altered by ±0.5mL/min), mobile phase composition (by using 85:15, MeOH: Water. The result of robustness study of the developed assay method was established in Tables. The result shown that during all variance conditions, assay value of the test preparation solution was not affected and it was in accordance with that of actual.

3.2.8. System Suitability. The system suitability tests represent an integral part of the method and are used to ensure adequate performance of the chromatographic system. The parameters, retention time (R_t) and peak area repeatability were evaluated different injections of the drugs.

The surveillance and results obtained from each validation experiment including specificity, linearity and range, LOD and LOQ, precision, accuracy, robustness, recovery, and system suitability lie well inside the acceptance criteria of ICH guideline. Since all the results are within the limit, the developed analytical method is considered as validated and suitable for probable use.

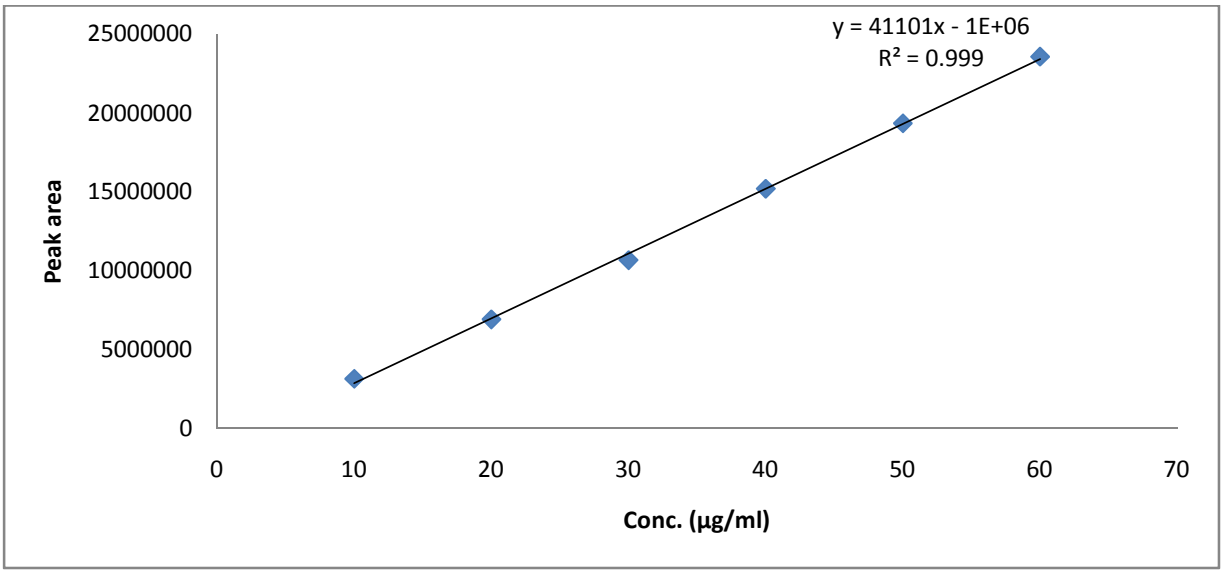


Fig: 1. Calibration curve of Zonisamide by HPLC

Table 3: Inter-day Precision Reading of Zonisamide by HPLC.

Sl. No.	Concentration (µg/ml)	Day	Peak Area	Mean=3150849
1.	10	Day 1	3150744	S.D=94.173
2.	10	Day 2	3150926	
3.	10	Day 3	3150877	R.S.D=0.0001%

Table 4: Intra-day Precision Reading of Zonisamide by HPLC.

Sl. No.	Concentration (µg/ml)	Peak Area	Mean=3114416
1.	10	3114206	
2.	10	3112021	S.D=2506.6
3.	10	3117021	R.S.D=0.08%

Table 5: Recovery Studies.

Formulation	Conc. Level (%)	Qty. spiked (µg/mL)	Qty. recovered (µg/mL)	Recovery (%)
Zonisep	50	20	19.49	97.45
Zonisep	100	40	38.92	97.30
Zonimid	50	30	28.26	94.20
Zonimid	100	60	59.64	99.40

Table 6: Robustness method of % of mobile phase.

Flow rate	Methanol: Water	Retention time	Area	SD value of Area	369818	RSD value of Area	2.3%
1.5(ml/min)	85:15	2.580	15723481				
1.5(ml/min)	Only methanol	2.053	15200479				

Table 7: Robustness method of flow rate (ml/min).

Flow rate	Methanol: Water	Retention time	Area	SD value of Area	6193	RSD value of Area	0.04%
1(ml/min)	Only methanol	2.567	15191720				
1.5(ml/min)	Only methanol	2.503	15200479				

Table 8: Assay result of the marketed formulation by the proposed method.

Formulation	Label claimed	Observed Amount	% Recovery
Zonisep	50mg	49.33mg	98.66
Zonimid	50mg	49.22mg	98.44

CONFLICT OF INTERESTS

The authors wish to confirm that there is no known conflict of interests associated with this paper. The authors confirm that they have given due consideration to the protection of intellectual property associated with this work and that there is no impediment to publication, including the trademarks mentioned in their paper.

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LINIARITY CHROMATOGRAM

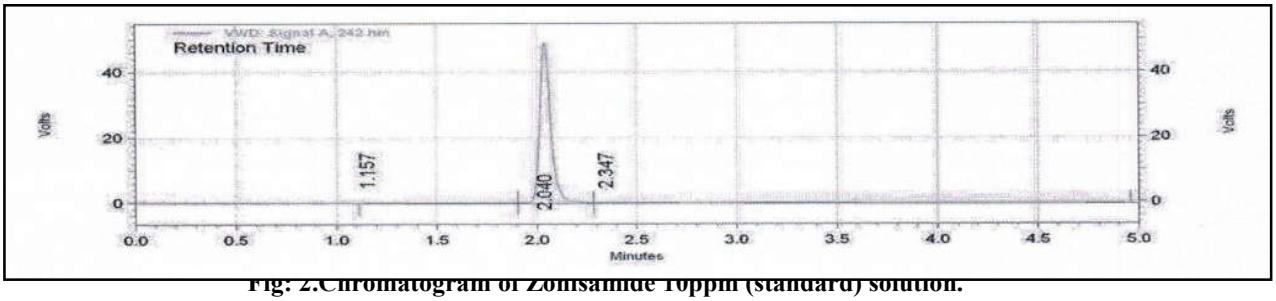

Fig: 2.Chromatogram of Zonisamide 10ppm (standard) solution.

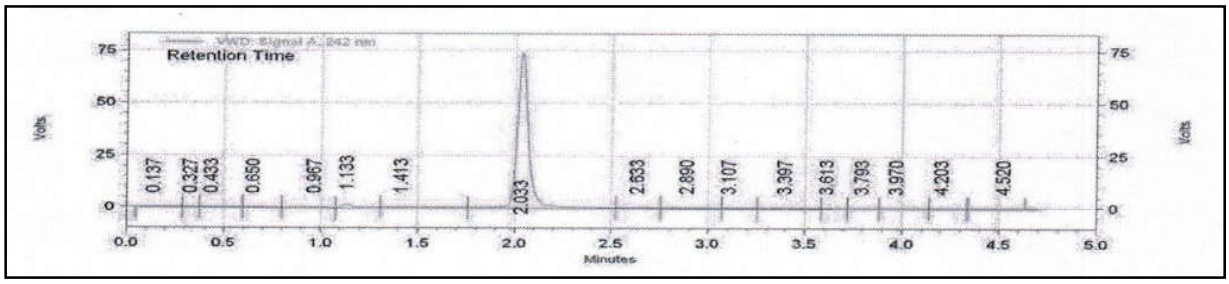

Fig: 3.Chromatogram of Zonisamide 20ppm (standard) solution.

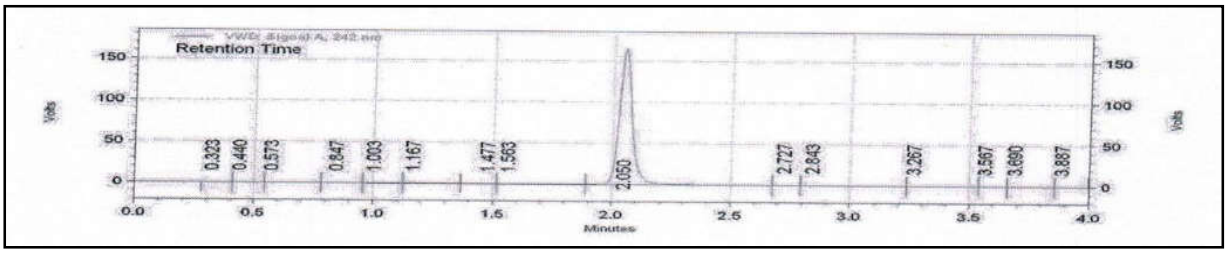

Fig: 4.Chromatogram of Zonisamide 30ppm (standard) solution.

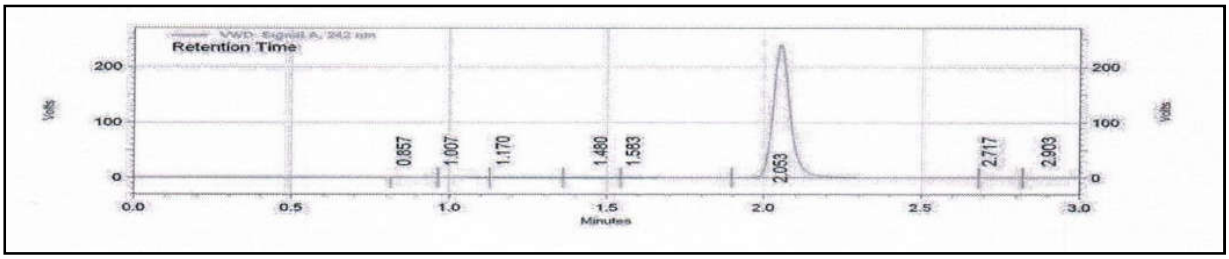

Fig: 5.Chromatogram of Zonisamide 40ppm (standard) solution.

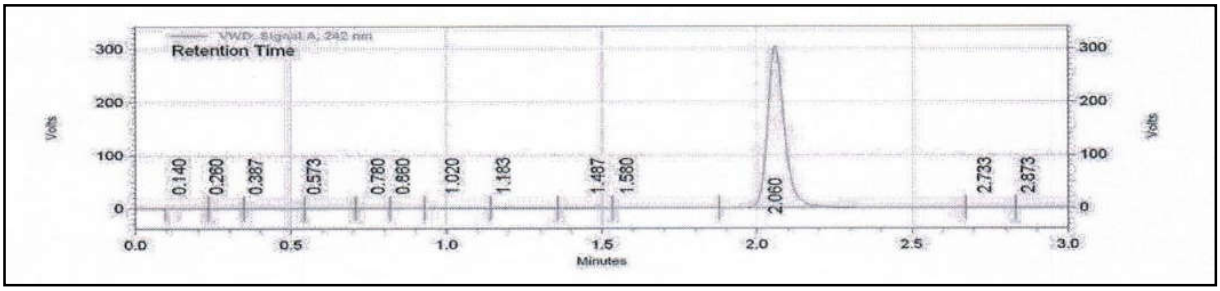

Fig: 6.Chromatogram of Zonisamide 50ppm (standard) solution.

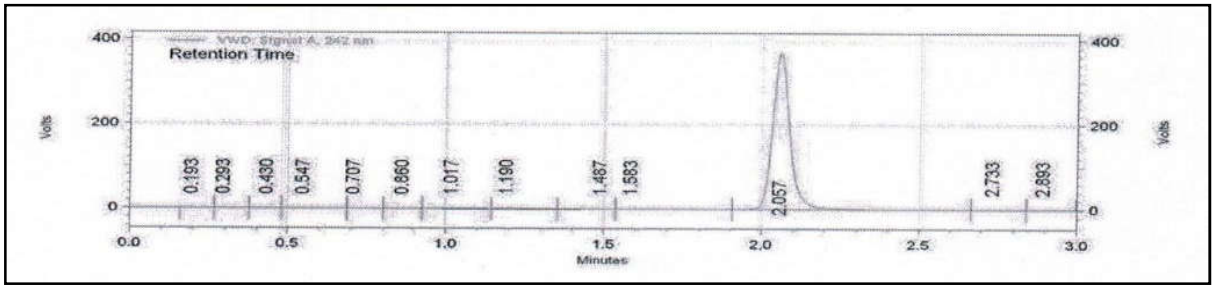

Fig: 7.Chromatogram of Zonisamide 60ppm (standard) solution.

INTER-DAY CHROMATOGRAM

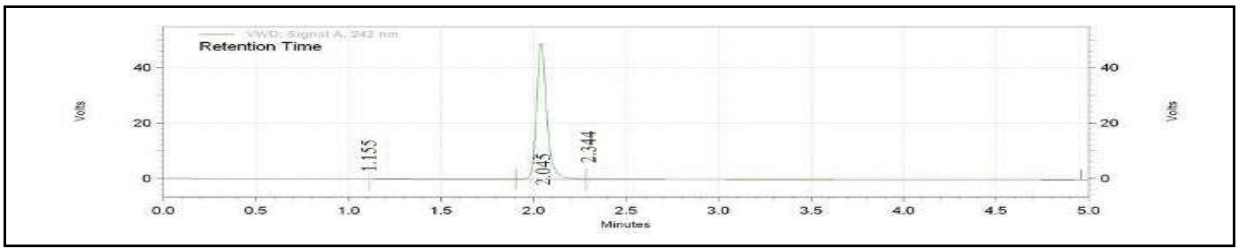

Fig: 8.Chromatogram of Zonisamide 10ppm standard solution (Day-1).

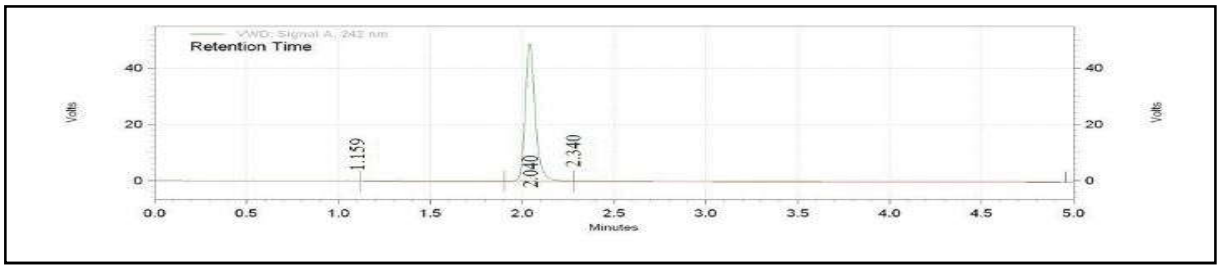

Fig: 9.Chromatogram of Zonisamide 10ppm standard solution (Day-2).

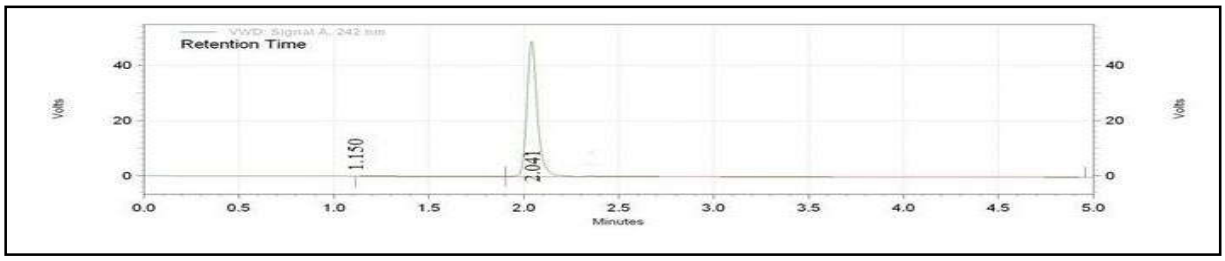

Fig: 10.Chromatogram of Zonisamide 10ppm standard solution (Day-3).

INTRA-DAY CHROMATOGRAM

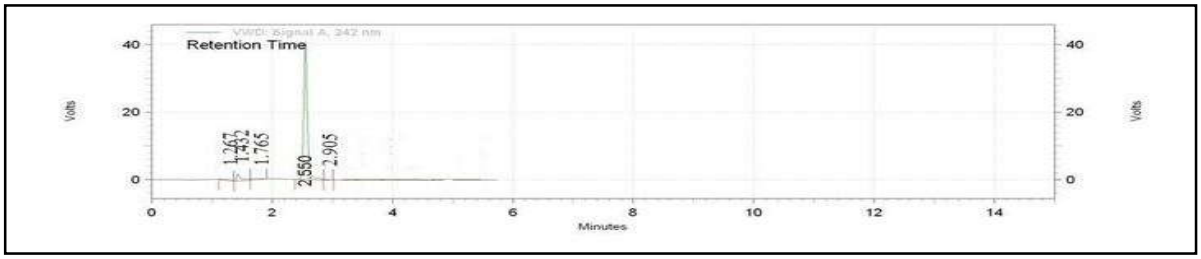

Fig: 11.Chromatogram ofZonisamide 10ppm standard solution (Intra-day).

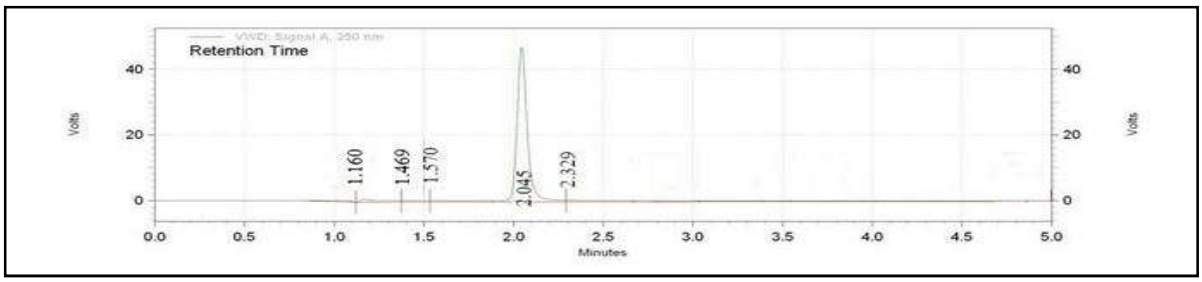

Fig: 12.Chromatogram ofZonisamide 10ppm standard solution (Intra-day).

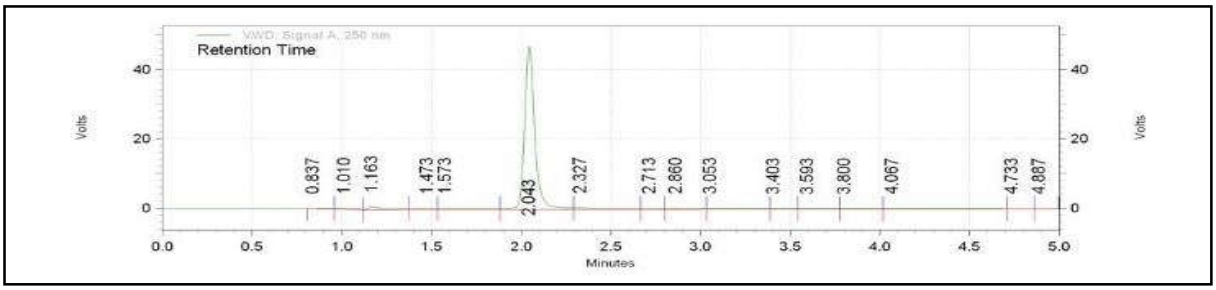

Fig: 13.Chromatogram ofZonisamide 10ppm standard solution (Intra-day).

RECOVERY CHROMATOGRAM

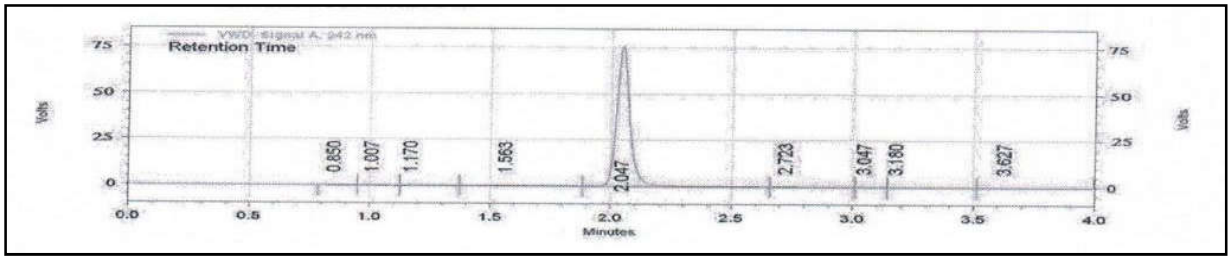

Fig: 14. Chromatogram of Zonisamide (standard+sample) 20ppm solution.

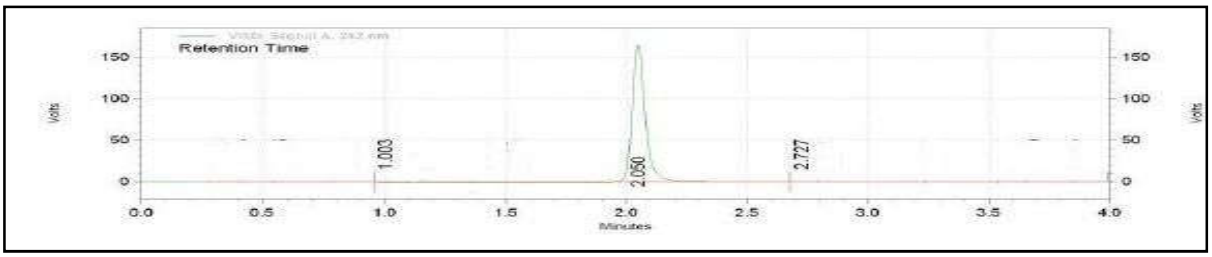

Fig: 15. Chromatogram of Zonisamide (standard+sample) 30ppm solution.

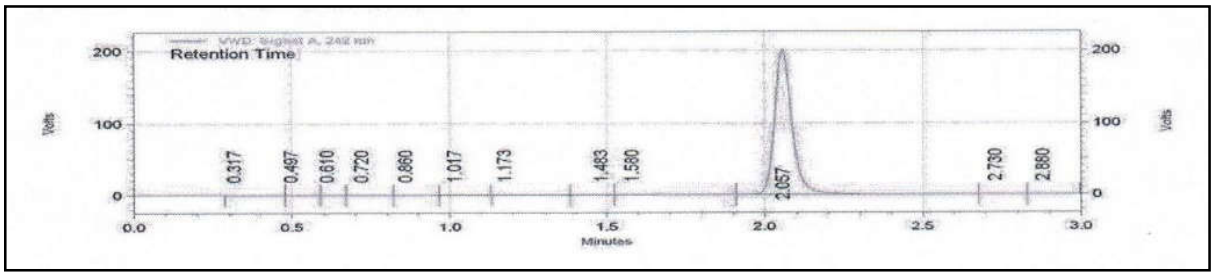

Fig: 16. Chromatogram of Zonisamide (standard+sample) 40ppm solution.

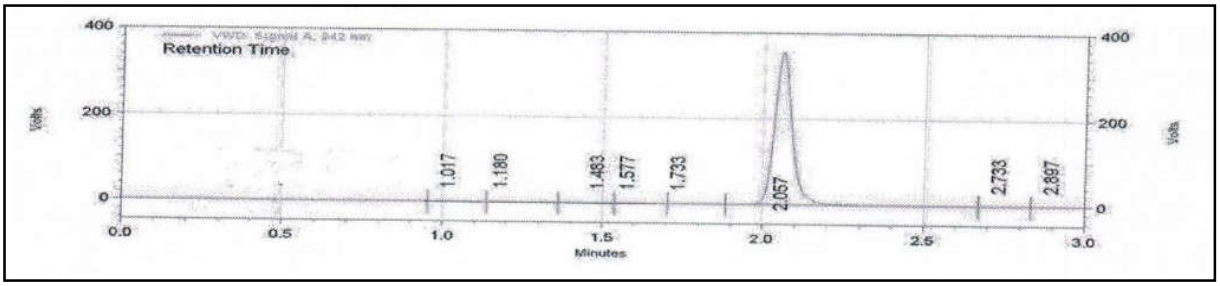

Fig: 17. Chromatogram of Zonisamide (standard+sample) 60ppm solution.

ROBUSTNESS CHROMATOGRAM

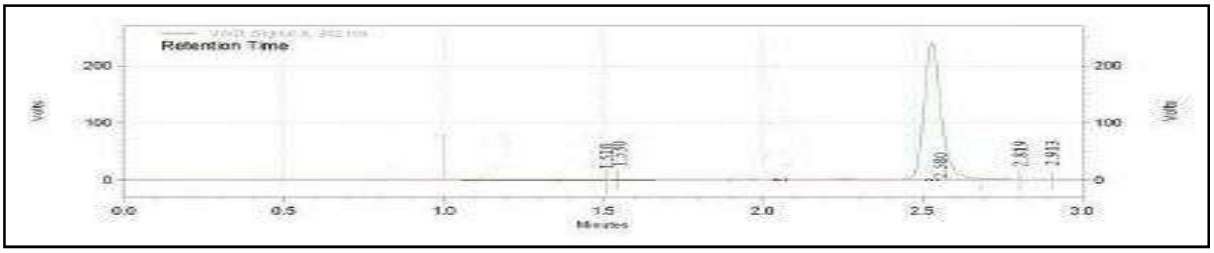

Fig: 18. Chromatogram of Zonisamide 40ppm (methanol: water, 85:15) solutio

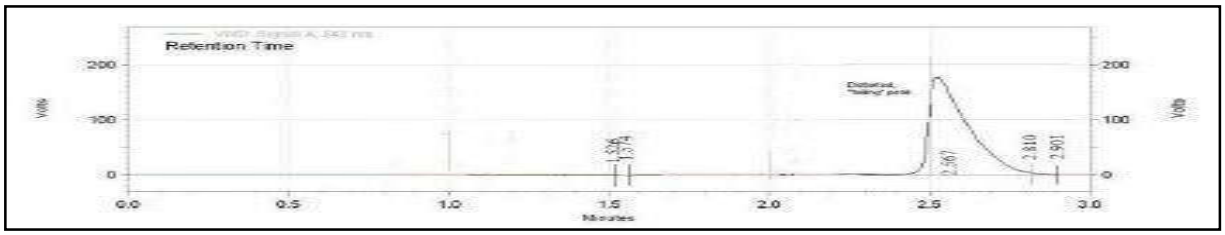

Fig: 19.Chromatogram of Zonisamide 40ppm solution (flow rate-1ml/min).

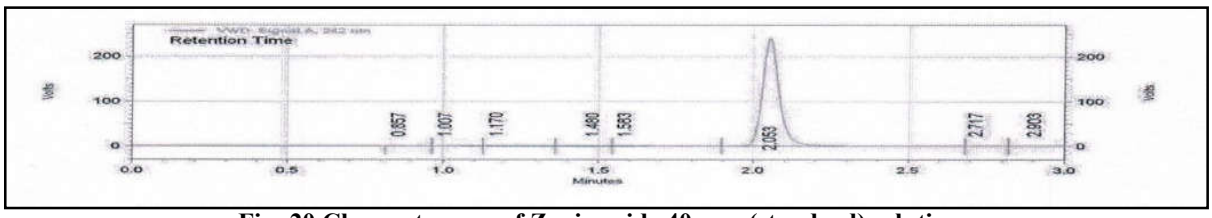

Fig: 20.Chromatogram of Zonisamide 40ppm (standard) solution.

SAMPLE (ASSAY) CHROMATOGRAM

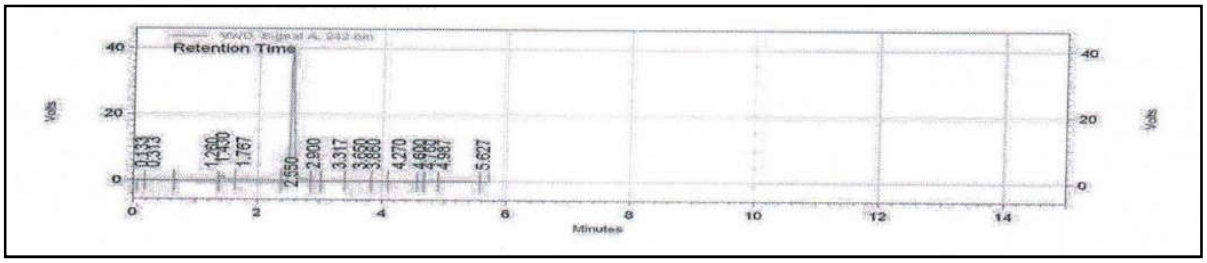

Fig: 21.Chromatogram of Zonisep (Zonisamide) 10ppm solution.

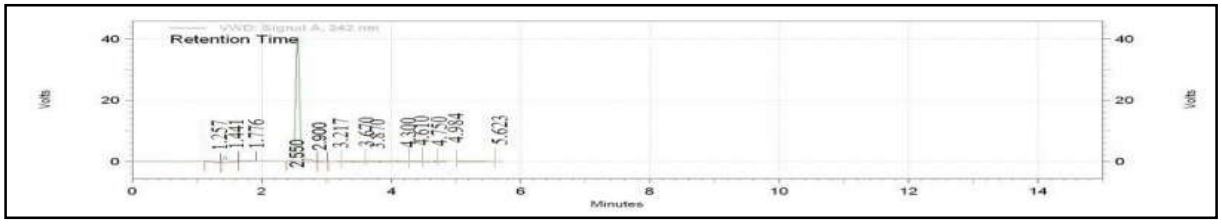

Fig: 22.Chromatogram of Zonimid (Zonisamide) 10ppm solution.

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