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METABOLITE ESTIMATION OF ANTI CONVULSANT DRUG IN MARKETED FORMULATION BY STRESS STUDIES EMPLOYING NOVEL CHROMATOGRAPHIC TECHNIQUE

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ABSTRACT

A novel rapid and accurate RP-HPLC technique has made it possible to precisely measure zonisamide, both in its pure form and in a tablet dosage form. Using a mobile phase consisting of 40 percent acetonitrile and 60 percent water (v/v), chromatography was performed on a Sunfire C18 (4.6 x 250mm, 5 m) column at a flow rate of 1.0 ml/min and detected at 254 nanometers (nm). The mean time that zonisamide lingered in the system was 2.165 0.02 minutes. The stress tests were performed in a variety of environments, including those that were acidic, alkaline, neutral, oxidative, UV, and hot. The drug-related peak was clearly isolated from the degradation-product-related peaks. Instability testing revealed that the drug degraded rapidly when exposed to air, acids, bases, heat, and light. The method yielded linear responses in the concentration range of 15-75 g/ml of Zonisamide, with a number of theoretical plates and tailing factor of 5846 and 1.31, respectively, and a correlation coefficient of 0.999. The assay for the medication came back at 100.80%. The process was shown reliable after it was checked and double-checked that all parameters were within the proper ranges. The suggested method for estimating zonisamide can be used for quality control testing of both bulk drugs and pharmaceutical formulations.

Keywords: Zonisamide, Stability-indicating RP-HPLC, Degradation, Validation.

INTRODUCTION

In the past, zonisamide was frequently used in conjunction with other antiepileptic drugs from generations for the treatment of partial-onset seizures[1-3]. This substance's official chemical name is 1,2-benzoxazol-3-ylmethanesulfonamide. Low solubility is observed for both chloroform and n-hexane. It can be broken down into smaller pieces by using one of the following solvents: methanol, ethanol, ethyl acetate, or acetic acid. It is unclear how zonisamide produces its effects; one possibility is that it blocks T-type calcium currents by binding allosterically to GABA receptors, while another is that it prevents the repetitive firing of voltage-gated sodium channels. The latter effect might lessen the uptake of glutamate and increase the uptake of GABA, two neurotransmitters involved in regulating nerve impulse transmission. Zonisamide is a new antiepileptic medication that shows promise for patients who have not found relief from other drugs. In 1989, it was given the green light for commercialization after long delays in Japan. The United States and much of Europe finally legalised it in 2000 and 2005, respectively (Fig. 1: Chemical structure of zonisamide).

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It is still unclear how exactly zonisamide works to prevent seizures. Its actions on sodium and calcium channels may, however, account for its antiepileptic benefits. Zonisamide stabilises neuronal membranes and inhibits neuronal hypersynchronization by blocking sodium channels and decreasing voltage-dependent, transient inward currents. However, it has no effect on L-type calcium currents[4].

Gas chromatography (GC)[5], micellar electrokinetic capillary chromatography (ECPC),[6,7] enzyme immunoassay[8], high-performance liquid chromatography (HPLC)[9-19], and their many permutations and combinations are all mentioned in the literature as methods for separating zonisamide. The measurement of its metabolites has only been reported in a small number of articles[14,17]. The author of this work zeroes in on the processes involved in the drug's involuntary breakdown and the subsequent purification of the active ingredient.

MATERIALS AND METHODS

Instrumentation

The Waters HPLC 2695 Alliance system equipped with a Sunfire C18, (250 mm x 4.6 mm, 5) column was used to create a high pressure liquid chromatographic method for quantitative measurement of Zonisamide. Auto-sampler and DAD 996 detector are built inside the device. The samples were injected via a rheodyne injector port with a capacity of 10 L. Empower 2 was used for the data analysis.

CHEMICALS AND SOLVENTS

Zonisamide's reference standard was a free sample generously donated by Sura Labs in Hyderabad, India. Market-formula Zonigran pills (Zonisamide 100 mg) were purchased at a pharmacy in the area. E.Merck (India) Ltd in Mumbai, India was used to get HPLC-grade water, methanol, and acetonitrile.

Chromatographic conditions

The most effective mobile phase for chromatographic separation of Zonisamide was determined to be HPLC-grade water and acetonitrile in a ratio of 60:40, V/V. Before usage, we sonicated the solvent combination and filtered it using a 0.45 membrane filter. At a rate of 1.0 mL/min, it was pumped through the column. The column was kept at 40 degrees Celsius, and the injection volume was 10 microliters. Before injecting the drug solution, the column was equilibrated by pumping the mobile phase through it for at least 30 minutes. The drug's presence was tracked at a wavelength of 282 nm. The scheduled duration was 5 minutes (Table 1: Optimized chromatographic conditions of Zonisamide and Fig. 2: Chromatogram of well resolved peak of zonisamide).

Preparation of mobile phase

After degassing in a digital ultrasonicator for 10 minutes, the mixture was filtered through a 0.45 filter under vacuum. The final volume was 400 ml (40%) Acetonitrile and 600 ml (60%) water.

Diluent Preparation

The Mobile phase was used as the diluent.

Preparation of Standard Solution

Carefully measure out 10 mg of the Zonisamide working standard and place it in a 10 ml clean, dry volumetric flask. Add around 7 ml of the Diluents and sonicate until the powder is totally dissolved.

To get a concentration of 45 g/ml, transfer 0.45 ml of one of the aforementioned Zonisamide stock solutions to a 10 ml volumetric flask, and then fill up to the mark with diluents.

Preparation of Sample Solution

We weighed 10 mg of zonisamide equivalent into a 10 mL clean dry volumetric flask and sonicated it to dissolve it fully, then added around 7 mL of diluent and brought the volume up to the mark with the same solvent.

The aforementioned stock solution can be further diluted to the appropriate concentration with the addition of diluent (0.45) before being utilised in an experiment.

Procedure

Inject the three replicate injections of standard and sample solutions and calculated the assay values using standard formulae.

Degradation Studies

Oxidation

Separately, 1 ml of 20% hydrogen peroxide (H2O2) was added to 1 ml of zonisamide stock solution. We heated the solutions to 60 degrees celsius for 30 minutes. After diluting the resulting solution to 45 g/ml, 10 ml were injected into the HPLC system, and chromatograms were recorded to determine the sample's stability.

Acid Degradation Studies

Zonisamide stock solution (1 ml) was refluxed in 2N hydrochloric acid (1 ml) for 30 minutes at 60 degrees Celsius. The resulting solution was diluted to 45 g/ml, and then 10 ml of the solution was put into the system and chromatograms were recorded to determine how well the sample had held up.

Alkali Degradation Studies
One millilitre of Zonisamide stock solution was mixed with one millilitre of two-percent sodium hydroxide and allowed to reflux at 60 degrees Celsius for thirty minutes. Sample stability was determined by diluting the resulting solution to a concentration of 45 g/ml, injecting 10 ml into the system, and recording the resulting chromatograms.

Dry Heat Degradation Studies
To test the effects of dry heat degradation, the standard medication solution was baked at 105° C for 6 hours. In order to determine the stability of the sample, HPLC analysis was performed by diluting the resulting solution to a 45 g/ml solution and injecting 10 ml into the system.

Photo Stability studies
The drug's photochemical stability was further investigated by subjecting a 1000 g/ml solution to UV light for 7 days at 200 watts per square metre in a photo stability laboratory. 10 l of the resulting solution was injected into the HPLC system to determine how stable the sample was, and the chromatograms were recorded at concentrations ranging from 45 g/ml to 0.01 g/ml.

Neutral Degradation Studies
The medication was subjected to stress testing under controlled settings by refluxing it in water for 6 hours at 60 degrees Celsius. In order to determine the stability of the sample, HPLC analysis was performed by diluting the resulting solution to a 45 g/ml solution, injecting 10 l into the system, and recording the chromatograms.

Method Validation[20-22]
Linearity
The concentrations of zonisamide in the final products were adjusted from 15 to 75 g/ml by diluting the standard solution with diluent to the mark in many 10 mL volumetric flasks. The medication was analysed using a UV detector set to 282 nm, and the peak area of each peak was recorded. The value of zonisamide's correlation coefficient was a perfect 1.000. An excellent association between peak area and drug concentration was found within the concentration range studied (Table 2: Linearity results).

Limit of detection and limit of quantification
We injected progressively lower amounts of the standard solution into the proposed HPLC technique to assess its limit of detection (LOD) and limit of quantitation (LOQ). Zonisamide's LOD was calculated to be 2.10 g/ml. Zonisamide has a lower detection limit of 6.50 ng/ml.

System suitability
Retention time, resolution, theoretical plates, and tailing factor were computed and compared to norms and ranges to assess the system's appropriateness.

Accuracy
Recovery studies of zonisamide in three dosage concentrations were used to evaluate the efficacy of the approach. Standard medication was added at 50%, 100%, and 150% concentrations to a fixed amount of sample that had been previously examined. There were three sets of each level. The amount of zonisamide in each pill was determined. Zonisamide was recovered with a mean yield of 100.06%, demonstrating that no excipients were interfering with the assay, and RSD of assay results below 0.05%, indicating that the procedure was accurate (Table 3: Recovery results of Zonisamide).
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Precision
Both the daily and weekly repeatability of zonisamide were analysed to establish its accuracy. An intraday precision evaluation found that zonisamide had a % RSD of 0.98% (limit %RSD 2.0%) after injecting a standard solution of fixed concentration at varying time intervals. The intra-day precision was also investigated by injecting the same concentration of standard solution on different days; the % RSD for zonisamide was 1.21% (limit%RSD 2.0%) (Table 4: Precision studies of Zonisamide).
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Ruggedness and Robustness
The experiment was run on a variety of instruments by a variety of operators using a variety of columns of the same kind to determine the method's robustness. By varying the chromatographic parameters slightly, we were able to evaluate the method's stability. There were no noticeable shifts in the chromatograms, proving the HPLC method to be stable and reliable.

Assay
The amount of each drug in the samples was determined by injecting 10 L of a standard and sample solution and analysing the peak area of zonisamide. Assay results showed that the concentration of zonisamide achieved 100.08% of the label claim (Table 5: Assay results of Zonisamide).

RESULTS AND DISCUSSION

Using a Sunfire C18 column (250 x 4.6 mm, 5) in isocratic mode and a mobile phase composition of water and acetonitrile in a ratio of 60:40, V/V, the Stability indicating RP-HPLC technique was improved with the goal of developing an accurate approach in tablet dosage form. In order to achieve a peak with desirable shape and resolution, a 60:40, V/V mixture of water and acetonitrile was used. The drug component was measured with an ultraviolet (UV) detector set to a wavelength of 282 nm, and the flow rate was set to 1.0 mL/min. Table 1 displays the outcomes of using optimal HPLC settings. Within a concentration range of 15–75 ng/mL, the technique was linear for Zonisamide (r = 0.999). Table 2 displays the linearity data, while Fig. 4 depicts the linearity curve. Zonisamide had a recovery rate of 100.06 percent, proving the validity of the approach. Table 3 displays the outcomes of various recoverability analyses. The intra-day and inter-day % RSD for Zonisamide were 0.98 and 1.21, respectively, indicating the technique is accurate. Table 4 displays the outcomes of accuracy studies. Commercial Zonisamide formulations were analysed using a validated approach. Table 5 displays the results of the % assay for Zonisamide, which was found to be 100.09%.The 2.165-minute retention period of Zonisamide was measured. The column performed well as measured by its tailing factor of 1.31 and its number of theoretical plates of 5846. Zonisamide was found to have a sensitivity of 2.10 ng/mL and a quantification limit of 6.50 ng/mL, respectively. Table 6 displayed a summary of the parameters used for validation and system appropriateness. Figures 2 and 3 display a typical chromatogram for the medication zonisamide. Zonisamide's reactivity to various stressors, such as 10% hydrogen peroxide, 0.1 M HCl, 0.1 M NaOH, heat, UV light, and neutral circumstances, was studied as part of the degradation experiments. Chromatograms with deteriorated peaks, as shown in Table 7, provide proof that degradation has occurred. Zonisamide was shown to be rather unstable for the majority of experimentally simulated stresses. The drug, when exposed to peroxide, emitted two metabolites, represented by two peaks in the chromatogram, which were themselves destroyed. Under acidic circumstances, the medication released five metabolites, represented by five peaks in the chromatogram's degradation phase. To a similar extent, the drug's degradation under basic conditions resulted in the production of three degradation products, shown by three degradation peaks in the chromatogram. Under heat, UV, and neutral conditions, however, the medication only produced a single peak of degradation metabolites in chromatograms.

Table 1: Optimized chromatographic conditions of Zonisamide

S. No.	Parameter	Condition
1	Mobile phase	Water:Acetonitrile (60:40)
2	Diluent	Mobile phase
3	Column, make	Sunfire C18 ((250 mm x 4.6 mm, 5µ)
4	Column temperature	40^0C
5	Wave length	282nm
6	Injection volume	10ul
7	Flow rate	1.0ml/min
8	Run time	**5** min
9	Retention time	2.165±0.02mins

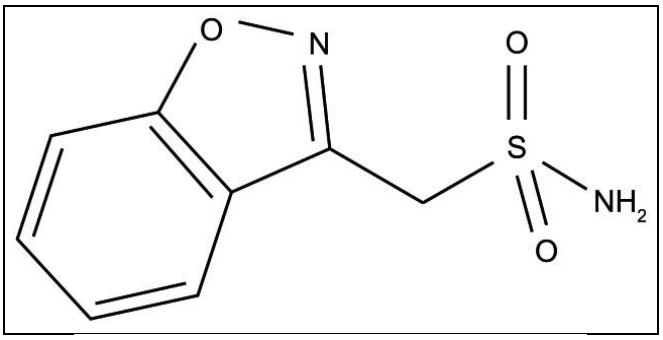

Fig. 1: Chemical structure of zonisamide

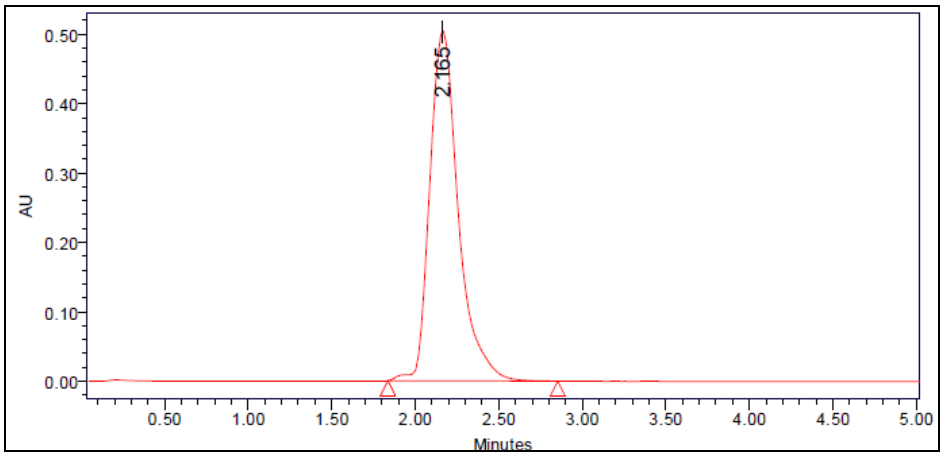

Fig. 2: Chromatogram of well resolved peak of zonisamide

Table 2: Linearity results

S. No.	Concentration	Area
1	15µg/ml	168581
2	30 µg/ml	354709
3	45 µg/ml	522589
4	60 µg/ml	696532
5	75 µg/ml	857576

Table 3: Recovery results of Zonisamide

Level	Amount added(µg/mL)	Amount found(µg/mL)	% Recovery	Mean recovery
50%	22.5	22.5102	100.50	100.06 %
100%	45.0	44.9868	99.30	
150%	67.5	67.5059	100.40	

Table 4: Precision studies of Zonisamide

Concentration (µg/mL)	Intra-day precision (%RSD)	Inter-day precision (%RSD)
45	0.98	1.21

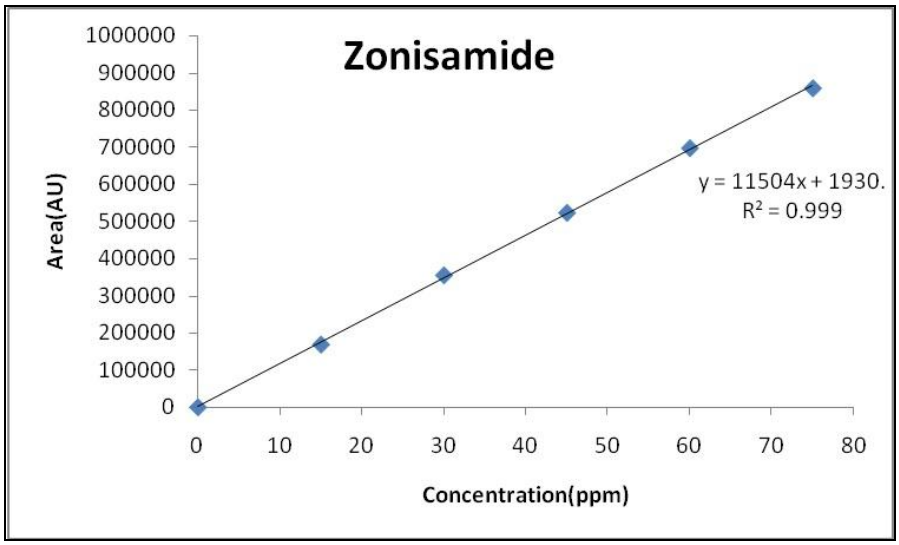

Fig. 3: Linearity curve of Zonisamide

Table 5: Assay results of Zonisamide

S. No.	Formulation	Label claim	Amount found	%Assay
1	Zonegran	100mg	100.09mg	100.09%

Table 6: Summary of validation parameters

S. No.	System suitability	Results
1	Linearity range (µg/mL)	15-75 µg/mL
2	Correlation coefficient	0.999
3	Theoretical plates (N)	5846
4	Tailing factor	1.31
5	LOD (µg/mL)	2.10 µg/mL
6	LOQ (µg/mL)	6.50 µg/mL
7	Regression Equation	Y=11504x+1930

Table 7: Degradation Data of Zonisamide

Stress Condition	Area of the drug peak	Number of degradant peaks	(%) Degradation	Active drug (%) after degradation
Optimized	522589	-	-	100.00
Acid (0.1 M HCl)	490607	5	6.12	93.88
Base (0.1 M NaOH)	486687	3	6.87	93.13
Peroxide (3 % H_2O_2)	477855	2	8.56	91.44
UV Conditions	512921	1	1.85	98.15
Thermal Conditions	515691	1	1.32	98.68
Neutral Conditions	517938	1	0.89	99.11

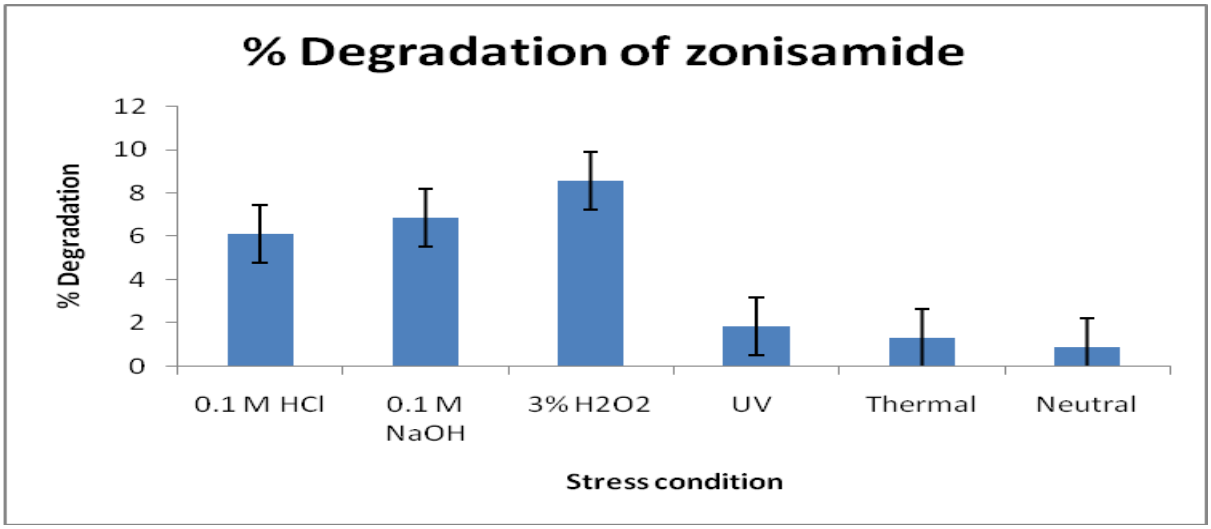

Fig. 4: Graphical representation of degradation of zonisamide

CONCLUSION

We designed and validated a rapid, simple, precise, selective, and sensitive RP-HPLC technique for zonisamide using UV detection as a stability indicator. This method can be used to rapidly and accurately determine zonisamide concentrations in both bulk materials and pharmaceutical formulations.

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