wjpmr, 2021,7(2), 196-200

SJIF Impact Factor: 5.922

WORLD JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.wjpmr.com

Research Article

ISSN 2455-3301

WJPMR

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR DETERMINATION OF ZONISAMIDE FROM TABLET FORMULATION

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DOI: https://doi.org/10.17605/OSF.IO/RGWJ4

Article Received on 04/12/2020 **Article Revised on 24/12/2020** **Article Accepted on 14/01/2021**

ABSTRACT

A new RP-HPLC method was developed for the rapid estimation of zonisamide from tablet formulation. The separation was carried out on Inertsil C_{18} ODS (4.6mm I.D x 250 mm) using mobile phase methanol and water (50:50, v/v) with retention time 4.71 min at flow rate 1.0 ml/min. at 238 nm. The validation of proposed method was performed in terms of linearity and range, accuracy and precision, and robustness. The drug showed the linearity over the concentration range from 1-5 µg/mL with r^2 0.998. Limit of detection and limit of quantitation was found to be 0.413µg/mL and 0.005 µg/mL, respectively. The average percentage recovery obtained for drug from tablet formulation was 99.17 to 101.04. In robustness, after slightly varying in chromatographic conditions, no significant change in response was recorded. Method was validated as per ICH guidelines.

KEYWORDS: RP-HPLC, Zonisamide, Tablet formulation, Validation.

1. INTRODUCTION

Zonisamide (1,2-benzisoxazole-3-methane sulfonamide) belongs to antiepileptic ccategory.[1] It is officially USP.[2] It used as an adjunctive antiepileptic in the treatment of refractory partial seizures in adults. The drug used as alone and in combination as well in the management of epileptic seizure.[3-4] There are several analytical method used for the determination of various drugs as a alone or in combination with other drugs from various pharmaceutical form.[5-25] In literature, there is report of Uv-vis spectrophotometry, HPLC and HPTLC method for determination of drug alone and in combination with other drugs.[26-31] However, all reported RP-HPLC method did not cover validation as per ICH. Validation studies which is essential to probe the chemical behavior of the molecule. Validation shows the test performs according to specifications when executed for the first time using the personnel, equipment, and reagents available.[32-33] Therefore, attempts were made to develop simple, accurate and rapid RP-HPLC method for the estimation drug from tablet formulation and method was validated as per ICH guidelines.

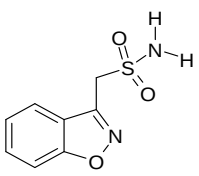

Figure 1: Zonisamide.

2. MATERIALS AND METHODS

2.1 Instrumentation and chemicals

Chromatography was performed with Youngline ACME 9000 (Autochro-3000 software) system coupled with Inertsil (4.6 mm I.D x 250 mm) C18 column and UV 730 detector. A Rheodyne injector (manual loading) with a 20 µL external loop was used. All chemicals and reagents used in method were of HPLC grade. Pure drug sample of zonisamide was kindly supplied by the Eisai Pharmaceuticals India Pvt. Ltd. Mumbai. Tablet (Zonegran®, 100mg) were purchased from local pharmacy shop.

2.2 Standard solution

An accurately weighed 100 mg of pure drug sample of zonisamide was dissolved in 100 mL of methanol with vigorous shaking. It was filtered through 0.45µ membrane filter. Concentration of final solution is 20 µg/ml.

2.3 Selection of wavelength

In order to determine the absorbance maxima various concentrations of the drug were prepared and scanned using UV absorption spectrophotometer for spectrum in the range of 200-400 nm.

2.4 Selection of mobile phase

For the selection of mobile phase, various solvents individually and in combinations were tried and a mobile

phase methanol and water in ratio of 50:50 v/v was selected for study, as the drug was eluted within a time period of 5 minutes with sharp peak.

2.5 Linearity and Range

Linearity of the method was studied by injecting five concentrations of the drug prepared in the methanol in the range 1-5 µg/ mL into the HPLC system. The peak areas were plotted against the corresponding concentrations to obtain the calibration graphs.

2.5 System suitability parameters

System suitability parameters were evaluated on standard stock solution of the solution was injected into the chromatographic conditions. Parameters studied to evaluate the suitability of system were retention time, area under curve, asymmetry, capacity factor and number of theoretical plates.

2.6 Optimized chromatographic conditions

The chromatographic conditions maintained throughout the experimental work were HPLC system (Youngline ACME 9000 LC System), detector (UV 730 detector), column (Inertsil (4.6 mm I.D x 250 mm) C18), mobile phase (methanol and water (50:50, v/v)), wavelength (238 nm), mode (isocratic), flow rate (1.0 ml/min), Injector (Loop system (20 µL)), temperature (ambient) and drug concentration (2 µg/mL).

2.7 Assay of tablets

Ten tablets of zonisamide were weighed and crushed to fine powder. On the basis of labeled claim, powder equivalent to 20 mg of drug was taken in 100 ml volumetric flask and was dissolved in about 20 ml methanol. The flask was shaken for 10 minutes and final volume was made up to 10 ml with methanol. The solution was then filtered through 0.45µ membrane filter and filtrate was used for analysis (200µg/mL). 1 ml of above solution was further diluted to 100 ml to get concentration 2 µg/mL.

2.8 Methodology

The above mentioned chromatographic conditions were set and mobile phase was allowed to equilibrate with the stationary phase as indicated by steady base line. Standard and sample solution were injected separately by loop injector and the chromatograms were recorded. The retention time (t_R), HETP, tailing factor (t_F) for drug were recorded. From the corresponding areas obtained in standard and sample chromatograms, the amount of drug was calculated

2.9 Validation of method

Studied validation parameters includes accuracy and precision, linearity & range, limit of detection & limit of quantitation and robustness.[34] Recovery study (accuracy) was carried out by addition of standard drugs solutions to preanalysed sample. Recovery study was undertaken at three levels i.e. 80%, 100% and 120%. Linearity was studied by injecting a series of dilutions of mixed standard stock solution in the concentration range 1-5 µg/ml into the HPLC system using 20µl volume. Calibration graph was plotted as concentration versus AUC to regression equation. The LOD & LOQ were confirmed by diluting known concentrations of drug until the average area under curve (AUC) were approximately 3 or 10 times the standard deviation of AUC of the blank for five replicate determinations. The signal/noise ratios 3:1 and 10:1 were taken as the LOD and LOQ, respectively. Robustness was studied by making changes in the chromatographic conditions, such as slight change in change in mobile phase flow rate (±0.1 ml/min), mobile phase composition (±1%), and change in wavelength (±1 nm). Percent contents of drugs were measured in preanalysed tablet formulation.

RESULTS AND DISCUSSION

Zonisamide, an antiepileptic drug was chosen to develop RP-HPLC method on the basis of literature survey and availability of analytical method. Solvent methanol was used to prepare standard and sample solution of the drug. Concentration selected was 2µg/mL and about 20 µL of each solution was injected for the analysis. mobile phase consisting of mixture of methanol and water (50:50 v/v) was selected as it gives sharp peak of drug at flow rate 1 mL/min detected at 238 nm with less retention time of 4.71 min which is less than 5 min. It denotes method is rapid. The low value of standard deviation after repeated injection of samples indicates system suitability parameters are stable over the given chromatographic conditions (Figure 1).

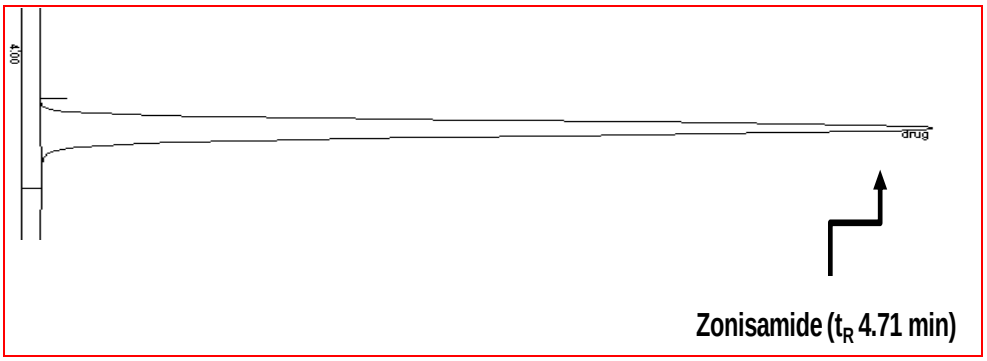

Figure 1: Zonisamide separated by-using mobile phase methanol & water at 1 ml/min detected at 238 nm.

Table 1: Results of the method.

Study	Parameters	Result	Study		Parameters	Result *
Lab. claim	Zonegran Tab.	100 mg	Robustness#	Flow rate	0.9 ml/min	101.62
% Recovery	80% level	99.17			1.0 ml/min	100.02
	100% level	101.05			0.1 ml/min	98.97
	120% level	98.94				
Linearity and range	Range	1-5 µg/mL		Mobile phase (a: b)	51:49	99.18
	% RSD*	0.95			50:50	99.97
	Slope	5.011			49:51	100.54
	R^2	0.998				
	LOD	0.413		WL	237	99.33
	LOQ	0.005			238	99.10
System suitability parameter	Ret. Time	4.71			239	99.98
	AUC	954		Column	Thermo C18	101.17
	HETP	6139			Vydac C18	99.09
	Tailing Factor	1.27			Inertsil C18	99.48

*Mean of three results; # % contents of drugs were measured in preanalysed tablet formulation; a- Methanol, b-water;

The validation study was performed as per ICH guidelines. Value of coefficient of correlation (r^2 0.998) reflects the method is linear over the studied concentration range. The percentage recovery of drug from formulation, close to 100%, and its low percent relative standard deviation values, indicates a high accuracy of the method. Obtained LOD and LOQ value indicated the method is sensitive and can be used for detecting low concentration of drug. % Recovery for both the drug was closed to 100% w/w. The percent contents of drugs were measured in preanalysed tablet formulation. Precision was determined by studying system suitability parameters by injecting standard solution. The results are expressed % RSD. The capacity of developed method was checked by performed robustness study. The conditions changed deliberately were change in flow rate (± 0.1), mobile phase composition (± 1), wavelength (± 1) different columns and percent contents in formulation were estimated. The result showed develop method remain unaffected. Results of experimental work are shown in Table 1.

CONCLUSION

The proposed RP-HPLC method is simple, sensitive, precise and accurate. Since the analysis is completed within 5 minutes, it clearly indicates that the method is rapid and thus it could be for routine analysis of zonisamide from bulk drug and its tablet dosage forms.

ACKNOWLEDGMENTS

Authors are thankful to Shri. Yogendra Gode, President, IBSS's Dr. Rajendra Gode Institute of Pharmacy, Amravati and Dr. Rajendra Gode College of Pharmacy, Amravati for providing excellent research facility to undertake this work. Authors are also thankful to Emcure Pharmacecals, Pune for providing gift samples of drugs.

Disclosure of conflict of interest

The authors declare no conflicts of interest.

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