Journal of Positive School Psychology http://journalppw.com
2022, Vol. 6, No. 7, 1449 – 1452

HPLC METHOD FOR THE ESTIMATION OF ZONISAMIDE IN ITS FORMULATION

[1]S.N. Meyyanathan, [2]Ruchitha Bandla, [3]B. Babu, [4]M. Kalaivani

[1]Department of Pharmaceutical Analysis, JSS College of Pharmacy, JSS Academy of Higher Education & Research, Ooty-643001, The Nilgiris, Tamil Nadu, India, snmeyyanathan@jssuni.edu.in
[23]Department of Pharmaceutical Analysis, JSS College of Pharmacy, JSS Academy of Higher Education & Research, Ooty-643001, The Nilgiris, Tamil Nadu, India
[4]Indian Pharmacopoeia Commission, New Delhi

Abstract

Zonisamide (ZON) is an anti epileptic used for adjunctive therapy of partial seizures in adults, and children (6 years and above). Many methods for the estimation of Zonisamide have been reported which include spectrophotometry and HPLC methods. A validated HPLC method has been developed for estimation of Zonisamide in its formulation. In HPLC method, a C18 column and methanol: acetonitrile : water in the ratio 60:30:10, pH adjusted to 3.0 using orthophosphoric acid were used at a flow rate of 0.8 mL/min and detected at 285 nm. The retention time for Zonisamide was found to be 3.6 min. The developed method was validated for linearity, precision, accuracy, specificity, LOD and LOQ as per ICH guidelines. Linearity was observed in the range of 5-25 µg/mL for Zonisamide and correlation coefficient was found to be 0.9954. LOD and LOQ for Zonisamide were found to be 0.11 µg/mL and 0.33 µg/mL respectively. The % recovery was found to be 99.8%. The method was applied for estimation of Zonisamide in its pharmaceutical dosage form. The assay result was found to be 99.5% of percentage label claim of Zonisamide.

Keywords: Zonisamide, High performance liquid chromatography, Capsules, Validation.

I. INTRODUCTION

Zonisamide (ZON) is an anti epileptic used for adjunctive therapy of partial seizures in adults, and children (6 years and above). Several methods for the estimation of Zonisamide have been described in the literature which include spectrophotometric method and high performance liquid chromatography (HPLC) methods [1-6]. The reported methods in the literature suffered from disadvantage of poor sensitivity and the present study reports the development and validation of a liquid chromatographic method with better detection ranges in pure form and its dosage forms. The developed method was validated for linearity, precision, accuracy, specificity, LOD and LOQ as per ICH guidelines.

II. MATERIALS AND METHODS

2.1. Materials

Methanol and Aceonitrile of HPLC grade by Sigma Aldrich, Ortho phosphoric acid and Sodium dihydrogen orthophosphate of AR grade by Rankem Fine Chemical Limited and Water from Milli-Q RO framework (Millipore, Bedford, USA) were used. Working standard of Zonisamide was obtained as a gift sample from Indian Pharmacopoeia Commission (IPC), New Delhi, India.

2.2. Instrumentation

Shimadzu Prominence HPLC system LC-20 AT-VP solvent delivery system (pump) Hamilton injector with 20 □l loop SPD M-

20AVP Photo Diode Array detector Lab solution CS software for data management was used. Isocratic separation was achieved using Shimpack C18 (250 x 4.6 mm i.d, 5µ) as a stationary phase and the mobile phase consists of methanol: acetonitrile : water in the ratio 60:30:10, pH adjusted to 3.0 using orthophosphoric acid were used at a flow rate of 0.8 mL/min and detected at 285 nm.

2.3. Preparation of standard solutions

ZON solution was prepared by dissolving accurately about 10 mg in methanol and making up the volume to 10 mL with methanol and this solution was refrigerated at 2-80C. From stock solution of ZON, aliquots of 0.5, 1.0, 1.5, 2.0 and 2.5 mL were transferred in series of 100 mL volumetric flask and diluted up to mark with methanol to get the concentration range of 5, 10, 15, 20 and 25 µg/mL of ZON.

2.4. Preparation of sample solution

Twenty capsules of marketed formulation of ZON (Brand Name : ZONISEP) were weighed accurately, separated powder and a weight of the powder equivalent to 50 mg of Zonisamide was transferred to 100 ml volumetric flask, dissolved the contents with methanol and filtered. The filtered solution was made up the volume with methanol to obtain a concentration of 500 □g/ml of Zonisamide. The above solution was further diluted with methanol to achieve a concentration of 10 □g/ml

2.5. Method Validation

Validation of the method for specificity, linearity, accuracy, precision, range, quantitation limit, and detection limit, robustness and system suitability as per the ICH guidelines.

2.5.1. Specificity

The analyte response measurement in the presence of excipients to demonstrate the specificity.

2.5.2. Linearity

The average of six determinations at five concentration levels covering the range of 5-25 µg/mL for ZON, the evaluation of linearity was performed.

2.5.3. Accuracy

Determination of accuracy was demonstrated for standard addition method from recovery studies according to ICH guidelines. The pre-analyzed samples were spiked with standard drug ZON.

2.5.4 Precision

Evaluation of precision was carried out by inter-day and intra-day comparison study samples consisted of the concentration (six replicates) of 10 µg/mL.

2.5.5. Limit of detection (LOD) and limit of quantification (LOQ)

Determination of LOD and LOQ was assessed by the signal-to-noise ratio. LOD ratio was 3:1 whereas LOQ of the drug could be quantified with minimum peak area in the ratio of 10:1.

2.5.6. Ruggedness and Robustness

The alteration in the condition of the experiment like operators, the source of reagents, similar type column and optimized conditions like pH, mobile phase ratio and flow rate were used as assessment tool for the ruggedness and robustness monitoring.

III. RESULTS AND DISCUSSION

3.1. Specificity

No peaks were eluted along with the retention time of ZON hence, the developed method was specific for the determination of ZON in the formulation (Figure 1).

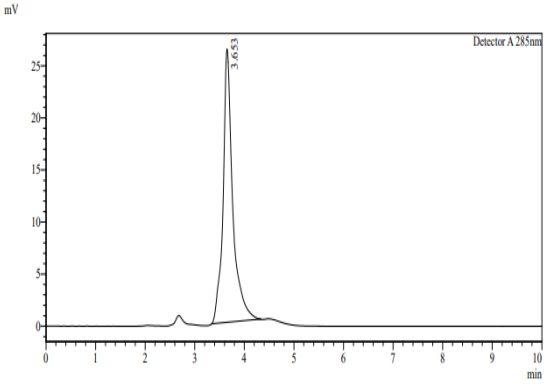

Figure 1: HPLC chromatogram of Zonisamide standard

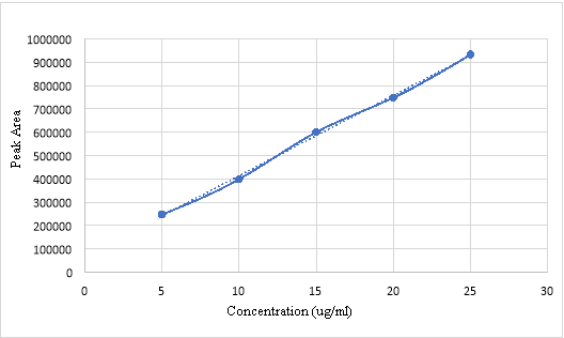

Figure 2: Calibration curve of Zonisamide

3.2. Linearity

The evaluation of the method to be linear was by six determinations at five concentration levels with a range of 5-25 µg/mL for ZON (Figure 2).

3.3. Accuracy

The accuracy of the method was carried for the quality control samples by standard addition method, and the accuracy was found to be 99.5 % (Table 1).

Table 1: Accuracy and Precision studies of Zonisamide

Sample (µg/ml)	**Amount found (µg/ml) ± SD***	**Intra day**		**Inter day**	
		Accuracy	**Precision (% RSD) ****	**Accuracy**	**Precision (% RSD) ****
10	9.95±0.15	99.2	1.17	99.0	1.23

*SD: Standard Deviation

**RSD: Relative Standard Deviation.

Table 2: Assay and recovery studies for Zonisamide formulation

Formulation	**Label claim**	**Amount taken for assay(µg/ml)**	**Amount found ±SD (N=6)**	**Found mg/cap**	**Recovery %**
S_1	50 mg	10	9.9± 1.15	49.95	99.8

*S1: Zonisamide Capsule from market

3.4. Precision

Calculation of the method for precision was carried by the intra-day and inter-day precision studies at the concentration of 10 µg/mL and they were found to be within the limits.

3.5. Limit of detection and Limit of quantification

The lowest limit detected for the method for IMA was at 0.11 µg/mL based on the signal-to-noise ratio 3:1. Due to the increase in the sensitivity of the method, quantification was done at 0.33 µg/mL for ZON.

3.6. Ruggedness and Robustness

When alteration in the condition of experiment was done, no notable changes in the parameters of chromatography were observed, proving that the developed method was found to be highly rugged and robust.

3.7. Assay

Estimation of Zonisamide in dosage forms by HPLC method was carried out using the optimized chromatographic conditions. The sample solution was prepared and the chromatogram was recorded. The percentage of the drug found in formulation, standard

deviation was calculated and presented in Table 2. The result of analysis shows that the amount of drug was in good agreement with the label claim of the formulation.

IV. CONCLUSION

An accurate, precise, rapid, selective and cost-effective method has been established for the quantitative estimation of selected drug in commercial formulation by HPLC. The developed and validated method is suitable for the analysis of ZON in commercial formulations.

CONFLICT OF INTEREST STATEMENT

The authors declare that there are no conflicts of interest

ACKNOWLEDGEMENT

Authors thank the Indian Pharmacopoeia Commission (IPC), New Delhi for providing the Zonisamide working standard as gift sample.

References

[1] Furuno K, Oishi R, Gomita Y, Eto K. Simple and sensitive assay of zonisamide in human serum by high-performance liquid chromatography using a solid-phase extraction technique. Journal of Chromatography B. 1994;656(2):456-9.
[2] Nakamura M, Hirade K, Sugiyama T, Katagiri Y. High-performance liquid chromatographic assay of zonisamide in human plasma using a non-porous silica column. Journal of Chromatography B. 2001; 755(1-2):337-41.
[3] Rao DV, Chakravarthy IE, Kumar SR. Stability indicating HPLC method for the determination of zonisamide as bulk drug and in pharmaceutical dosage form. Chromatographia. 2006;64(5):261-6.
[4] Hosseini M, Alipour E, Farokhsir A. Determination and validation of zonisamide and its four related substances by HPLC and UV-spectrophotometry. Indian Journal of Pharmaceutical Sciences. 2010;72(3):302-6.
[5] Lourenço D, Sarraguça M, Alves G, Coutinho P, Araujo AR, Rodrigues M. A novel HPLC method for the determination of zonisamide in human plasma using microextraction by packed sorbent optimised by experimental design. Analytical Methods. 2017;9(40):5910-19.
[6] Gaikwad B, Wani R, Bairagi SH. Development and validation studies of Zonisamide by RP-HPLC method in capsule dosage form. World Journal of Pharmacy and Pharmaceutical Sciences. 2017;6(9):1480-87.