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DETERMINATION OF ZONISAMIDE IN CAPSULE DOSAGE FORM BY USING RP-HPLC

K. ANANDA REDDY*

Department of Pharmaceutical Chemistry, Nirmala College of Pharmacy, Buddayapalli, KADAPA – 516002 (A.P.) INDIA

ABSTRACT

A simple and cost effective, fast and precise reverse phase high performance liquid chromatographic method is described for the determination of zonisamide in pure form and in pharmaceutical formulations. This method is based on using a Luna 5µ C_{18} column and the size of coloum is 5 micron, 250 x 4.60 mm from phenomenex. Mobile phase is methanol: phosphate buffer (pH adjust to 3.5 ± 0.1 with ortho-phosphoric acid), the ratio of mobile phase is 65 : 35 v/v, the flow rate is 1 mL / min, and effluent was monitored at 285 nm. The elution time was 8.5 min. The linearity range was 10-60 µg/mL for zonisamide.

Key words: Zonisamide, RP-HPLC, C_{18}, Tablet.

INTRODUCTION

Therapeutics and Technology Assessment Subcommittee and Quality Standards Subcommittee of the American Academy of Neurology and the American Epilepsy Society concluded that zonisamide was effective in reducing seizure frequency as adjunctive therapy in adult patients with refractory partial seizures, but that there were not enough studies to support a recommendation for its use in children[1]. Zonisamide (1, 2-benzisoxazole-3-methane sulfonamide (Fig. 1) is used as an anticonvulsant in patients with epileptic disorders. The precise mechanism of zonisamide's antiepileptic effect remains undefined. It has been suggested that zonisamide raises the seizure threshold through action at sodium and calcium channels, stabilizing neuronal membranes and suppressing neuronal hypersynchronization[2,3]. Several methods have been reported for the analysis of zonisamide using gas chromatography (GC)[4], micellar electrokinetic capillary chromatography[5,6] enzyme immunoassay[7], high performance liquid chromatography (HPLC) with UV detection using solid phase extraction[5] and HPLC method only for zonisamide by different methods[8-15].

*Author for correspondence; E-mail: kareddy@live.com

Reported methods involved complicated time-consuming multi-step liquid-liquid extraction techniques. To the best of our knowledge, there is no work in the literature reported about the estimation of zonisamide from pharmaceutical formulation by using RP-HPLC. The purpose of this investigation was the development of a rapid, sensitive and validated HPLC method for quantification of zonisamide from capsule forms.

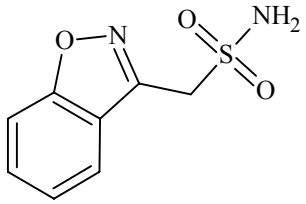

Fig. 1: Chemical structures of zonisamide

EXPERIMENTAL

Materials and method

Standard zonisamide was obtained from Dr. Reddy's Laboratories, Hyderabad. Potassium dihydrogen phosphate AR, ortho-phosphoric acid AR salts HPLC grade and methanol HPLC grades were supplied by S.D Fine Chemicals, Mumbai. Water HPLC grade was obtained from a milli-QRO water purification system.

A gradient high-pressure liquid chromatography (Shimadzu HPLC Prominence UFLC Series) with LC-20AT Prominence Pumps, variable wavelength programmable UV/Vis SPD-20 A Prominence Detector, SIL-20 AC HT/ Prominence UFLC auto Sampler (Shimadzu) and operating software LC Lab Solution. The method was carried on a Phenomenex Luna 5 µ C_{18} (250 * 4.60 mm i.d, 5 µ) column as a stationary phase. The mobile phase consisted of 0.02 M potassium dihydrogen phosphate salt (pH adjusted to 3.5 ± 0.1 with ortho-phosphoric acid) as aqueous phase and methanol. The mobile phase was filtered through a 0.45 µ membrane filter and degassed before analysis. Methanol and aqueous phase in the ratio of 65 : 35 v/v was used as the mobile phase at a flow rate of 1 mL/min. A SIL-20 AC HT/ Prominence UFLC auto Sampler was used for the injection of sample. Detection was done at 285 nm and separation was carried out at the room temperature of about 20^{o}C.

Standard stock solution of the drug was prepared by dissolving 25 mg of zonisamide in a mixture of methanol : water (1 : 1 v/v) and made up to with 25 mL with the same (1000 µg/mL). Working standard solution was prepared by diluting 1 mL of the stock solution to 10 mL with methanol: water (1 : 1 v/v) (100 µg/mL). The gradient dilution were prepared by

taking 1, 2, 3, 4, 5 and 6 mL of solution and made up to 10 mL with methanol : water (1 : 1 v/v) solution. Twenty µL of the solution from each flask was used for experiment. Calibration curve was constructed by plotting mean peak area against the corresponding drug concentration (Fig. 2). The detector response was found to be linear in the concentration range of 10-60 µg/mL (Table 1). The typical chromatogram of zonisamide drug solution is shown (Fig. 3). Calibration curves could be represented by the following equation y = 7923x - 47002 (R^2 = 0.998). This equation was used for the determination of zonisamide from capsules.

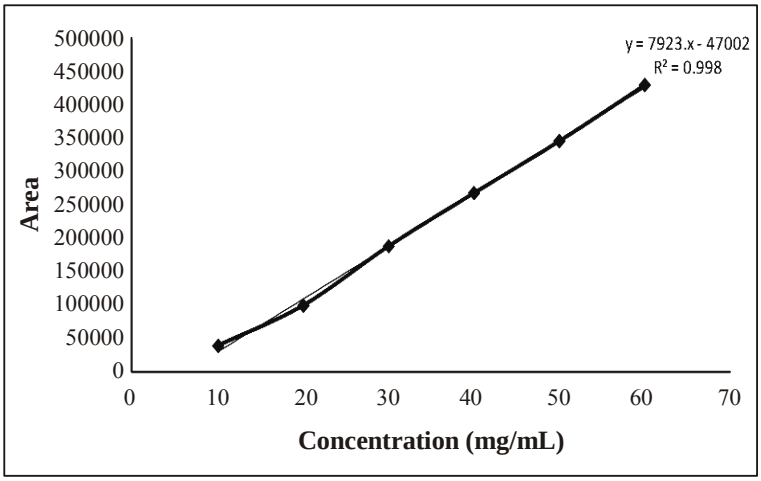

Fig. 2: Zonisamide standard plot (Concentration vs area)

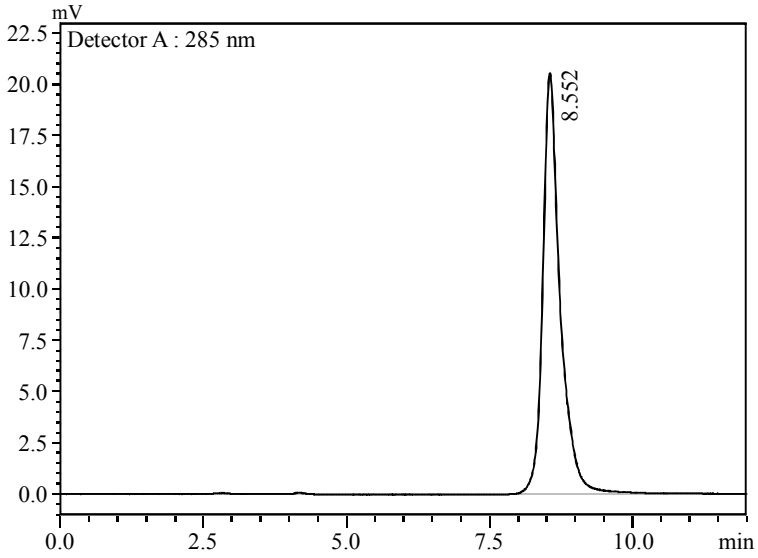

Fig. 3: Chromatogram of zonisamide drug solution

Table 1: Detector response (Concentration vs area)

Concentration (µg/mL)	1	2	3	Average area
10	41128	41223	41321	41224
20	102143	99947	102478	101522
30	191421	189947	190144	190504
40	269527	270381	269707	269872
50	358849	357090	359522	347812
60	431309	431937	432003	431750

For the estimation of drug from commercial formulation, 20 Capsules of two brands-Zonisamide capsule (Dr. Reddy's Laboratories Limited, Hyderabad.) and Zonisep capsule (Sun Pharmaceuticals Industries Ltd.) were taken each contained 100 mg and 25 mg of zonisamide were finely powdered. A quantity equivalent to 25 mg was transferred into 25 mL volumetric flask, dissolved and made up to with methanol : water (1 : 1 v/v) solution. The solution was filtered through a 0.45 µ membrane filter. One milliliter of the resulting solution was then diluted to 10 mL with an above used solution. From this, 0.5 and 1 mL sample was taken and their volume was made up to 10 mL each.

RESULTS AND DISCUSSION

A chromatogram of these solutions was obtained by injecting 20 µL of each sample in to the chromatographic system (Fig. 4). There was no interference from diluents and lubricants. The retention time of the drug was 8.5 min. Chromatographic parameters such as peak asymmetry (A) and capacity factor (k) were found to be 1.10 and 0.81, respectively. To study the accuracy, reproducibility and precision of the proposed method, recovery experiments were carried out. A fixed amount of the pre-analyzed sample was taken and standard was added at three different levels. Each level was repeated five times. The summaries of recovery studies are reported in Table 2.

The present study comprises a high performance liquid chromatography method to determine zonisamide from capsules dosage form. Experiment was carried out to establish the method. The mobile phase, bearing methanol : phosphate buffer in proportion of (65 : 35) was found to be idal. The retention time of zonisamide was found 8.5 min. The value of percent recovery and standard deviation indicate that this method is accurate, reproducible, and precise. The summaries of final results are illustrated in Table 3.

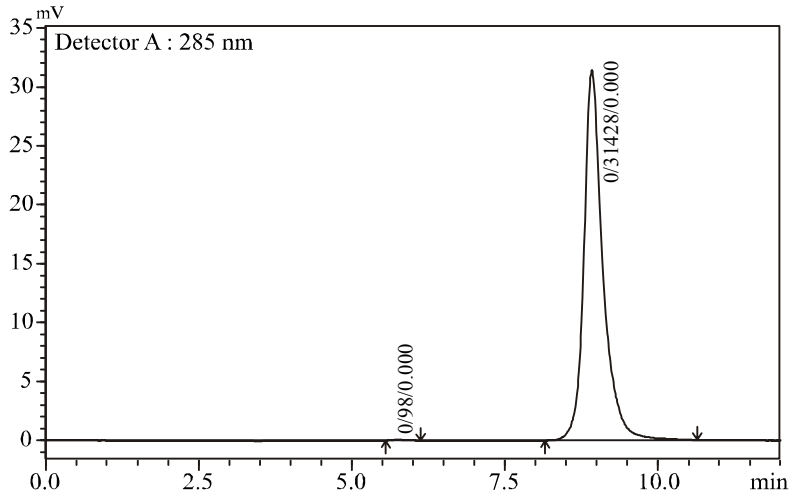

Fig. 4: Chromatogram of the sample capsule solution

Table 2: Summary of recovery studies

Drug zonisamide	Label claim tablet (mg)	Amount found (mg)	Recovery studies		
			Amount added (mg/mL)	Amount recovery (mg/mL)	Percentage recovery (%)
Tablet A	10	9.97 ± 0.025	5	15.1 ± 0.05	100.6
			10	20.5 ± 0.11	101.5
Tablet B	10	9.96 ± 0.025	5	15.1 ± 0.11	100.6
			10	20.2 ± 0.11	101.1

Tablet A is zonisamide capsule (Dr. Reddy's Laboratories Limited, Hyderabad) and
Tablet B is zonisep capsule (Sun Pharmaceuticals Industries Ltd.)

Table 3: Summaries of final result

Brand name	Amount found (mg/tablet)	% RSD	Percentage assay
Zonisamide capsule (Dr. Reddy's Laboratories Limited, Hyderabad)	9.97 ± 0.025	0.250	99.7
Zonisep capsule (Sun Pharmaceuticals Industries Ltd.)	9.96 ± 0.025	0.251	99.6

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