WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.990
Volume 4, Issue 9, 2272-2280. Research Article ISSN 2277– 7105

DEVELOPMENT AND VALIDATION OF SPECTROPHOTOMETRIC METHOD FOR ESTIMATION OF TOPIRAMATE BULK AND PHARMACEUTICAL DOSAGE FORM

Sivakameswari Somi Reddy*, Suresh Kannan.V, Nagaraju P.T., Dr.Venugopal. K, Murali Krishna. N.V, Ramadevi. R

Department of Pharmaceutical Analysis, Nirmala College of Pharmacy, Buddayapalle, Kadapa– 516002 (A.P), India.

Article Received on
20 July 2015,

Revised on 11 Aug 2015,
Accepted on 02 Sep 2015

***Correspondence for Author**
Sivakameswari Somi Reddy
Department of Pharmaceutical Analysis, Nirmala College of Pharmacy, Buddayapalle, Kadapa– 516002 (A.P), India.

ABSTRACT

A simple, specific, accurate and precise Spectroscopy method was developed and validated for the estimation of Topiramate in pharmaceutical dosage forms. The Standard solution was prepared by weighing 100 mg of Topiramate in 100 ml volumetric flask with methanol. The final Standard solution was made to produce 1000 µg / ml with methanol. Further dilutions were prepared as per procedure and were scanned at 260 nm. The linearity was found in the concentration range of 1-6 µg / ml. The Correlation coefficient was 0.999. The regression equation was found to be Y = 0.482 X - 0.035. The method was validated for linearity, accuracy, precision, limit of detection, limit of quantitation and ruggedness robustness. The limit of detection and limit of quantitation for estimation of Topiramate was found to be 0.010 (µg / ml) and 0.032 (µg / ml), respectively. The percentage recovery of Topiramate was found to be in the range of 100.36 ± 0.081 - 100.81 ± 0.055. Proposed method can be successfully applied for the quantitative determination of Topiramate in pharmaceutical dosage forms.

KEYWORDS: Topiramate; Method validation; UV-Spectroscopy; ICH guidelines..

INTRODUCTION

Topiramate, Anti convulsants, Anti-obesity agent, Neuroprotive agent[1] is a chemically is 2,3,4,5-Bis-O-(1-methylethylidene)-beta-D-fructopyranose sulfamate. (fig.1) it is a blocks the action[2] potentials elicited repetitively by a sustained depolarization of the neurons in a

time-dependent manner, suggesting a state-dependent sodium channel blocking action. Topiramate also augments the activity of the neurotransmitter gamma-aminobutyrate (GABA) at some subtypes of the $GABA_A$ receptor (controls an integral chloride channel), indicating a possible mechanism through potentiation of the activity of GABA. Topiramate also demonstrates antagonism of the AMPA/kainate subtype of the glutamate excitatory amino acid receptor. It also inhibits carbonic anhydrase (particularly isozymes II and IV), but this action is weak and unlikely to be related[3] to its anticonvulsant actions. Based on the literature survey it shows that very few analytical methods have been reported for the estimation of Topiramate which includes LC/MS/MS,[4,5,6] HPLC[7,8,9,10] (RP-HPLC/ELSD),UV.[11]

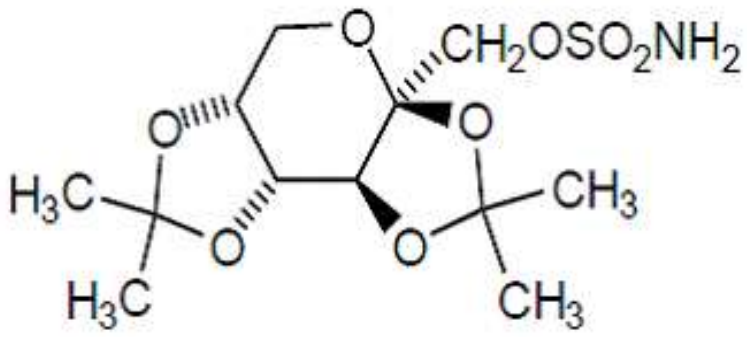

Fig.1: Structure of Topiramate

MATERIALS AND METHODS

1. Materials

Table No: 1 Materials used in present research work

S. No.	Materials	Source
1	Topiramate	Fine chemicals, Hyderabad.
2	Methanol	Thermo fisier scientntific, Mumbai.
3	Water	Qualigens, Mumbai.

2. Equipments

Table No: 2 Equipments used in present research work

S. No.	Equipment	Source
1	UV Spectrophotometer	Elicosl SL 210, Mumbai
2	Sonicator	Wensar

Method

UV Spectrophotometry

Experimental: ELICO SL 210 UV / Vis double beam Spectrophotometer with 1 cm matched quartz cells was used for all spectral measurements. All chemicals used were of A.R. grade. Authentic drug sample of Topiramate was given as a gift sample by Fine Chemicals, Hyderabad. Tablets of Topiramate were procured from local market.

Method Development

Solvent selection: In order to select suitable solvent for determination of Topiramate, various solvents were selected for the solubility studies and it was found that Topiramate was freely soluble in the following solvents; Methanol, Ethanol…etc. In the present investigation Methanol was selected as solvent.

Using Methanol: UV Spectrophotometric method involves in the determination of Topiramate in bulk drug and pharmaceutical formulations and has an absorption maximum at 260 nm in Methanol. It obeys Beer's law in the concentration range of 1-6 µg / ml.

Standard solution: 100 mg of Topiramate was dissolved in methanol in a 100 ml of volumetric flask and the solution was made up to volume with methanol.

Procedure: The standard solution of Topiramate was subsequently diluted with methanol to obtain a series of dilutions containing 1, 2, 3, 4, 5, 6 µg of Topiramate in 1 ml solution. The absorbance of these solutions was measured in Elico-SL 210, UV-Vis Spectrophotometer at 260 nm using methanol as blank. The concentration of Topiramate and the corresponding absorbencies are given in Table No.1. The absorbencies were plotted against concentration of Topiramate as shown in Fig 1. The concentration of the unknown sample was determined from the calibration graph. The regression equation and correlation coefficient were determined and are given in Table: 4.

Sample preparation of Topiramate: 20 tablets of Topiramate were weighed and powdered in glass mortar and the powder equivalent to 10 mg of Topiramate was weighed accurately and transfer into a 100 ml standard volumetric flask. The contents were dissolved in Methanol and sonicated for few minutes. This solution was filtered through (0.45microns) membrane filter. 1 ml of the filtrate was diluted with Methanol to get the concentration of 10 µg / ml.

Validation of Spectrophotometric method: 1. Accuracy: Accuracy is the closeness of the test results obtained by the method to the true value. To study the accuracy, 20 tablets of Topiramate were taken, and the powder was used to carry out the analysis. Recovery studies were carried out by addition of standard drug solution (15, 20, 25 µg / ml) to the sample at 3 different concentration levels and results were presented in Table: 5.

2. Precision: The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple samplings of homogenous samples. It provides an indication of random error results and was expressed as coefficient of variation (CV).

Intra and inter-day precision: A variation of results within the same day (intra-day), variation of results between days (inter-day) was analyzed and was shown in Table: 6. Intra-day precision was determined by analyzing Topiramate for three times in the same day at 260 nm. Inter-day precision was determined by analyzing the drug daily once for three days at 260 nm.

3. Linearity: The linearity of the method was demonstrated over the concentration range of 1-6 µg / ml of the target concentration. Aliquots of 1, 2, 3, 4, 5, and 6 µg / ml are prepared from standard solution; calibration curve was plotted and presented in Fig: 3.

4. Ruggedness: The solutions were prepared and analyzed with change in the analytical conditions like different laboratory conditions and different analyst and are given in Table: 7.

5. Robustness: The solutions were prepared and analyzed with change in the analytical conditions like different wave lengths are given in Table: 8.

Table No: 3. Calibration curve for Topiramate.

S. No.	Concentration (µg/ml)	Absorbance at 260 nm
1	0	0
2	1	0.409
3	2	0.939
4	3	1.423
5	4	1.854
6	5	2.359
7	6	2.896

RESULTS AND DISCUSSION

UV Spectroscopy

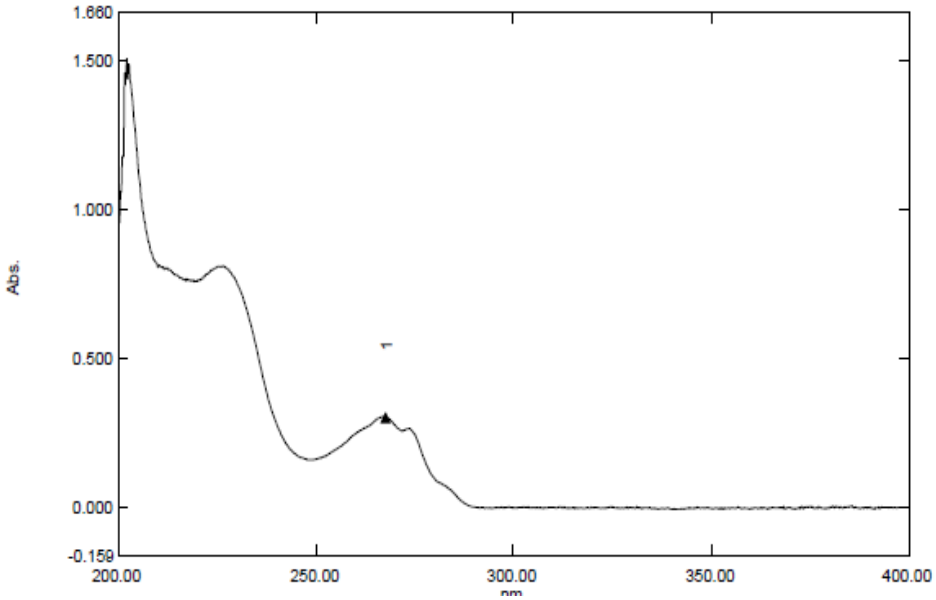

Fig No: 2. Spectra of Topiramate

Table No: 4. Optimum conditions, Optical characteristics and Statistical data of the Regression equation

Parameters	UV Spectroscopic Method
λ_{max} (nm)	260
Beer's law limits (µg / ml)	1-6
Regression equation (Y*)	Y = 0.482X - 0.035
Slope (b)	0.482
Intercept (a)	- 0.035
Correlation coefficient (r^2)	0.999
Limit of Detection (µg / ml)	0.010
Limit of Quantitation (µg / ml)	0.032

*Y= b C + a where C is the concentration of Topiramate in µg / ml and Y is the absorbance at the respective $\lambda_{max.}$ **Average of six determinations.

Table No: 5. Accuracy results for Topiramate at 260nm by UV Spectroscopy

Brand used	Amount of sample (µg / ml)	Amount of drug added (µg / ml)	Amount Recovered	% Recovery ± SD**
Qudexy-xr	15	15	15.1	100.66± 0.035
Qudexy-xr	15	20	20.16	100.80 ± 0.055
Qudexy-xr	15	25	25.09	100.36±0.081

**Average of six determinations.

Table No: 6. Precision results for Topiramate at 260 nm by Spectroscopy

Conc. µg/ml	Inter-day Absorbance Mean ± SD**	% CV	Intra-day Absorbance ± SD**	% CV
1	0.405 ± 0.00173	0.42	0.404± 0.00208	0.49
2	0.945 ± 0.0036	0.38	0.943 ± 0.00173	0.18
3	1.425 ± 0.00115	0.08	1.425 ± 0.00251	0.17
4	1.847 ± 0.00251	0.13	1.854 ± 0.00264	0.14
5	2.272 ± 0.00152	0.06	2.272± 0.00115	0.05

**Average of six determinations

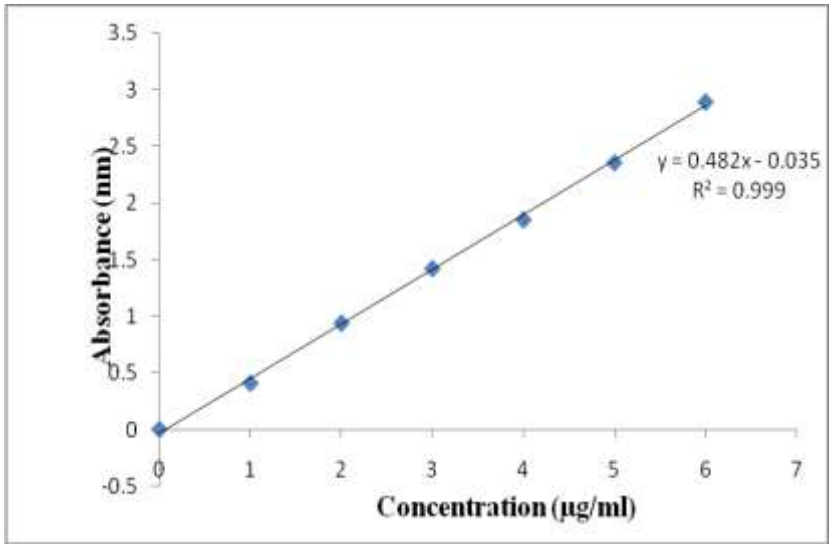

Fig No: 3. Linearity curve for Topiramate at 260 nm by Spectroscopy

Table No: 7. Ruggedness results for Topiramate at 260 nm by Spectroscopy

Brand name	Label claim (mg)	Analyst I		Analyst II	
		Amount found (mg)	%Recovery ± SD**	Amount found (mg)	%Recovery ± SD**
Qudexy-xr	25	24.92	99.68 ± 0.07	24.97	99.88 ± 0.08

** Average of six determinations.

Table No: 8. Robustness results for Topiramate at 260 nm by Spectroscopy

S.No.	Condition	Modification	Mean absorbance ±SD	%RSD (for absorbance)
1	Wavelength (nm)	258(nm)	0.402±0.001732	0.402
		232(nm)	0.398±0.001	0.398

** Average of six determinations.

Table No: 9. Limit of detection results for Topiramate at 260 nm by Spectroscopy

S. No.	Slope	S.D	LOD
1	0.464	0.0015	0.010

Table No: 10. Limit of quantitation results for Topiramate at260 nm by Spectroscopy

S. No.	Slope	S.D	LOQ
1	0.464	0.0015	0.032

The absorption spectra were recorded in the wavelength region of 200 - 400 nm in UV method. Topiramate showed linearity in the concentration range of 1 - 6 µg / ml in Spectroscopy methods respectively. The spectra are presented as Fig no:2. Beer's law range was confirmed by the linearity of the calibration curve of Topiramate, which were represented in Fig no: 3.

The optical characteristics such as absorption maxima, Beer's law limits, Molar absorptivity, Sandell's sensitivity, slope (b), intercept (C), correlation coefficient (r^2) obtained from different concentrations, percent relative standard deviation, LOD and LOQ values were presented in Table no: 4. The results showed that these methods have reasonable precision.
The quantitative estimation was carried out on formulation. The quantitative results obtained were subjected to statistical analysis to find out standard deviation and standard error values. The % RSD values are less than 2 indicating the precision of the methodology and low standard error values indicates the accuracy of the method. The statistical data's are given in Table no: 5.

Results obtained for the proposed methods confirm the suitability of these methods for Pharmaceutical dosage forms. The other active ingredients and excipients usually present in the Pharmaceutical dosage forms did not interfere in the estimation, when commercial dosage forms were analyzed by these methods. The Accuracy of the methods was confirmed by the recovery studies, by adding known amount of the pure drug to the formulation and the analytical data are presented in Table no: 5. the percentage recovery was found to be between 100.36 - 101.80 % shows that the method was free from the interference of excipients used in the formulation.

The Precision of an analytical method was calculated by performing intra-day and inter-day precision studies. The values were found to be precise and were presented in Table no: 6.

The results obtained in Ruggedness and Robustness test expresses the precision of the method. The Ruggedness results were listed in Table no: 7 and the Robustness results were shown in Table no: 8 respectively.

CONCLUSION

Development of methods to achieve the final goal of ensuring the quantity of drug substances and drug products is not a trivial undertaking. The capabilities of the four methods were complementary to each other. Hence they can be regarded as simple, specific and sensitive methods for the estimation of Topiramate in Bulk drug and Pharmaceutical dosage forms.

A very few analytical methods appeared in the literature for the determination of Topiramate. In view of the above fact simple sensitive, accurate, precise and economical analytical methods are planned to develop.

The UV Spectrophotometric method demonstrated applicable to the estimation of Topiramate in Bulk drug as well as in existing Pharmaceutical dosage form. In order to ensure that the data generated is accurate and precise. The experiments have been performed on calibrated equipments using suitable reference standards. The results found to be good and summarized in Table No: 3-10. In addition to positive requirements for analytical methods the striking advantage of all the presently developed methods are economical.

This method is validated in terms of accuracy, precision, repeatability, ruggedness and can be used for the routine determination of Topiramate in Bulk drug and Pharmaceutical formulations.

ACKNOWLEDGEMENT

We would like thank to Fine Chemicals, Hyderabad for providing reference sample of Topiramate respectively to facilitate this work and also to the Principal Dr.K.Venugopal, and Pharmaceutical Analysis Department lecturers of Nirmala College of Pharmacy, Kadapa for providing facilities during my experiment.

BIBLIOGRAPHY

1. www.drugs.com/topamax.html.
2. En. Wikipedia.org/wiki/Topiramate.
3. www.R_X list.com/Topmax-drug.html.
4. N. T. Ramarao, S. Vidyadhara, R. L. C. Sasidhar, B. Deepti, R. Surendra Yadav. Development and Validation of LC-MS/MS Method for the Quantification of Chiral Separated R-Bicalutamide in Human Plasma. American Journal of Analytical Chemistry. 2013; 8(4): 63-76.

5. Das Ganesh Kumar. Estimation of Topiramate in Human Plasma Using LC-MS/MS Method. Asian Journal Pharm Clinical Research. 2013; 6(1): 3217-220.
6. Popov TV, Lea Cvitkovi Mari.Helena Prosen and Darinka Brodnjak Von.ina. Determination of Topiramate in Human Plasma Using Liquid Chromatography Tandem Mass Spectrometry. Acta Chimica Slovenica. 2013; 60(1):144–50.
7. Viswanath Reedy, Useni Reddy Mallu, Pingili Sunil Reddy, K. Hussain Reddy, Maheswara Reddy, Musirike. RP-HPLC/ELSD Method Determination of Topiramate in Pharmaceutical Products. International Journal of Science Innovation and Discoveries.2011; 1(2):126-33.
8. Bahami G, Mohammadi B. Sensitive Analytical Method for Topiramate in Human Serum by HPLC with Pre-Column Fluorescent Derivatization and Its Application in Human Pharmacokinetic Studies. Journal of Chromatography B, Analytical Technologies Biomedical and Life Sciences. 2004; 850(1-2): 400-4.
9. Mercolini L, Robeto, Mario Amore. Simultaneous HPLC-F Analysis of three Recent Antiepileptic Drugs in Human Plasma. Journal of Pharmaceutical and Biomedical Analysis. 2010; 53(1): 62-67.
10. Bahami G, Mohammadi B. A Novel High Sensitivity HPLC Assay for Topiramate, Using 4-Chloro-7-Nitrobenzofurazan as Pre-Column Fluorescence Derivatizing Agent. Journal of Chromatography and Analytical Technology.2007; 4(1):400-4.
11. Bahrami G, Mirzaeei S, Mohammadi B, Kiani. A High Performance Liquid Chromatographic Determination of Topiramate in Human Serum Using UV Detection. Journal of Chromatography B, Analytical Technologies Biomedical and Life Sciences. 2005; 822(1-2): 322-25.