International Journal of ChemTech Research
CODEN (USA): IJCRGG, ISSN: 0974-4290, ISSN(Online):2455-9555
Vol.10 No.7, pp 843-849, 2017

Method Development and Validation by RP-HPLC for Estimation of Topiramate in Bulk and Pharmaceutical Dosage form

Mahadev B Kshirsagar*, Moreshwar P. Mahajan, Sanjay D. Sawant.

Department of Quality Assurance Technique, Smt. Kashibai Navale College of Pharmacy Kondhwa (Bk.) Pune-411048, India.

Abstract : A simple, precise, reliable, rapid and reproducible reversed-phase high performance liquid chromatographic (RP-HPLC) method has been developed and validated for the estimation of Topiramate(TPM). Chromatography was carried out Younglin (S.K) Gradient System UV Detector on C18(4.6X250 mm) column with a mobile phase composed of Methanol: Distill water (90:10 v/v) at a flow rate of 1 ml/min. The pH of mobile was adjusted by 0.05% ortho phosphoric acid (pH-3). Detection was carried out using a UV detector at 263 nm.Parameters such as linearity, precision, accuracy, ruggedness, LOD and LOQ were studied as per the ICH Q2(R1) guidelines. The retention times of TPM was4.35min. The linearity range for Topiramate 10-50µg/ml. The correlation coefficients of Topiramate was found to be 0.999. Developed method was found to be accurate, precise, selective and rapid for simultaneous estimation of Topiramate in pharmaceutical dosage forms. The proposed method can be useful in quality control of bulk manufacturing and pharmaceutical dosage forms.

Keywords : Topiramate, method validation, RP-HPLC, ICH guidelines.

1. Introduction

Topiramate (TPM) (Fig.1) is chemically2,3:4,5-Bis-O-(1-methylethylidene)-β-D-fructopyranose sulfm.Topiramate is used to treat epilepsy in children and adults, and it was originally used as an anticonvulsant. In children, it is indicated for the treatment of Lennox-Gastaut syndrome, a disorder that causes seizures and developmental delay. The drug is also used to treat migraines due to the effect it has on the blood vessels in the brain.

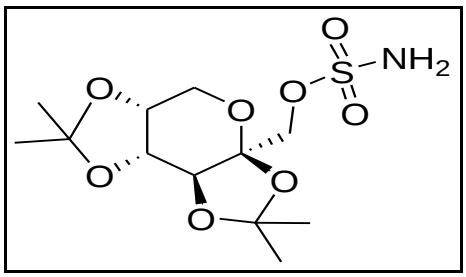

Fig.1: Structure of TPM

Topiramate is available in tablet dosage forms containing50mg.Literature survey shows that two UV spectroscopic[1,2] and four HPLC[3,4,5,6]methods for estimation of TPM in its bulk form. bulk and pharmaceutical dosage forms. The purpose of this work was to develop a simple, basic, rapid and economic RP-HPLC method for the determination of TPM in bulk and pharmaceutical dosage forms so as to provide better scope for further research on the drugs. The proposed method was optimized and validated as per the International Conference on Harmonization (ICH) guidelines Q2(R1)[7].

2. Materials and Methods

2.1 Instrumentation

Younglin(S.K) Gradient HPLC system and C_{18} column (250 x 4.6 mm) was used. The instrument is equipped with an UV 730 D detector. A 20µL rheodyne injector port was used for injecting the samples.

2.2 Chemicals and solvents

The working standard of Topiramate was obtained as gift samples from Encore Pharmaceutical Ltd, Aurangabad, India. The tablet market formulation was procured from local market. HPLC grade water was purchased from Qualigens Ltd, Mumbai,India. Methanol (HPLC Grade) was obtained from E.Merck (India) Ltd, Mumbai, India.

2.3 Chromatographic conditions

Column	: C18 column (250 x 4.6 mm)
Mobile phase	: Methanol: Distill water in proportion of 90:10 v/v (pH-3)
Detector	: 263 nm
Injection volume	: 20 µl
Flow rate	: 1 ml/min
Temperature	: Ambient
Run time	: 10 min
Diluents	: ACN

2.4 Selection of Mobile Phase

Standard solution of Topiramate (TPM) was injected into the HPLC system and run in different solvent systems. Mixture of different solvents were tried in order to determine optimum chromatographic conditions for effective separation of TPM. After several permutation and combination, it was found that mixture of Methanol: Water gives satisfactory results as compared to other mobile phases. Finally, the optimal composition of the mobile phase Methanol: Water in the ratio of 90:10(v/v) (pH-3) was selected, as it gave high resolution of TPM with minimal tailing.

2.5 Preparation Mobile Phase

Mobile phase was prepared by mixing 90ml of Methanol with 10ml of Distilled water .The pH of mobile was adjusted by 0.05% ortho phosphoric acid (pH-3).This mobile phase was filtered through 0.45µ membrane filter and then it was ultrasonicated for 30 minutes.

2.6 Preparation of standard stock solution

About 10 mg TPM are accurately weighed & transferred to 10 ml volumetric flasks. The drug is dissolved in 5 ml of ACN with shaking and then volume was made up to the mark with the mobile phase to get 1000µg/ml of TPM of standard stock solution of drug. Then it was ultrasonicated for 10 minutes and filtered through 0.20µ membrane filter.

- **Mobile phase:** Methanol: Water 90:10(v/v) (pH-3).
- **λ max:**263nm
- **Flow rate:**1ml/min.
- **Retention Time:** 4.35min.

2.7 System suitability parameters

The System suitability parameters were studied and the results are summarized in Table no.1

Table 1: System suitability parameters

Sr. No.	Parameters	TPM
1.	Retention time (min)	4.35
2.	No. of theoretical plate (N)	21861
3.	Tailing factor	1.17

Table 2: standard calibration curves

Sr. No.	Conc. (µg/ml)	Mean peak area
1	10	568.44
2	20	1034.52
3	30	1583.24
4	40	2113.00
5	50	2612.00

2.8 Study of beers-Lambert's law

Preparation of standard calibration curves and selection of analytical concentration ranges:

From standard stock of 1000µg/ml of TPM appropriate aliquots were transferred to a series of 10 ml volumetric flasks. The volume was made up to mark with mobile phase to get set of solutions having concentration range 10-50µg/ml for TPM. The diluted solutions were filtered through 0.2µ membrane filter. The filtrate (20µl) was injected into the column and chromatogram was recorded using optimized chromatographic conditions. The corresponding chromatograms were recorded and area of each peaks for TPM Are measured at 263 nm. Each sample solution was chromatographed in five times and mean peak area of TPM was calculated. Standard calibration graph for TPM are given in Fig no.3

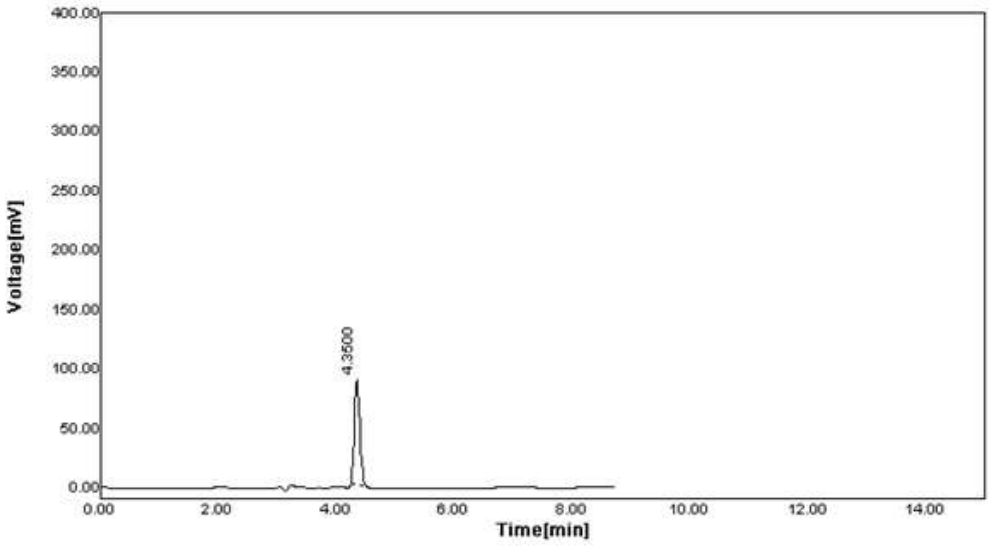

Fig.2: Optimized chromatogram of TPM

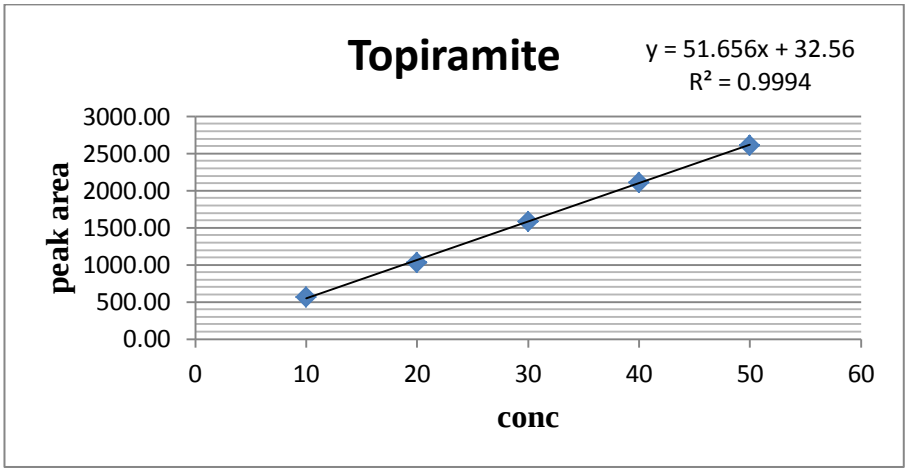

Fig. 3: Calibration curve of Topiramate by HPLC

3. Analysis of marketed formulation

For the estimation of drugs in the commercial formulations, twenty tablets containing 30mg of TPM are weighed and average weight was calculated. The tablets were crushed and powdered in glass mortar. For the analysis of drugs, quantity of powder equivalent to 10mg was transferred to a 10ml volumetric flask containing 5ml of mobile phase and then ultrasonicated for 20 min. Finally the volume was made up to the mark with mobile phase. The solution was filtered through Whatman filter paper no.42.This solution was further diluted with mobile phase to obtain mixed sample solution containing 30µg/ml of TPM. The solution was filtered through 0.20 µ membrane filter. A 20 µl of sample solution was injected to into sample injector for six times under chromatographic condition as described above. Areas of each peak were measuredat263nm.The amount of each drug present in the sample was determined from peak area of TPM present in the pure mixture respectively. The results are given in the Table no. 3 and Table no. 4.

Table 3: HPLC Assay of TPM (n=3)

Amt. taken (µg/ml)	**Peak Area**	**Amt. found (µg/ml)**	**% of drug found**
30	1598	30.20	100.68
30	1601	30.36	101.20
30	1595	30.25	100.83

Table 4: Statistical evaluation of marketed formulation

Drug	**%Mean***	**S.D.**	**%RSD**
TPM	101.60	0.34	0.33

**Average of six determination*

4. Validation

The developed method was validated as per ICH guidelines.

4.1 Linearity and Range:

The linearity of measurement was evaluated by analyzing different concentrations of the standard solutions of TPM. Beer's law was obeyed in the concentration range 10-50 µg/ml for TPM respectively.

Table 5: Linear regression data for calibration curves

Parameters	**Topiramate**
Linearity range	10-50 µg/ml
r^2	0.999
Slope	51.65
Intercept	32.56

$r^{2:}$ Correlation coefficient; S.E.: Standard error

4.2 Precision

4.2.1 Repeatability

To check the degree of repeatability of the method, six samples of the marketed formulation were analyzed. The results of the Repeatability are given in Table no. 6.

Table 6: Result of repeatability

Drug	**Conc. (µg/ml)**	**Area mean***	**Amount found***	**% Amount found***	**S.D.***	**%RSD***
TPM	20	1080.3	20.22	101.13	3.95	0.366

**Average of six determination*

Table 7: Results of Intra-day and Inter-day precision studies

Parameter	**Conc. (µg/ml)**	**Area mean***	**Amount found***	**% Amount found***	**S.D.***	**%RSD***
Intraday	20	1080.00	20.27	101.39	2.08	0.19
	30	1593.00	30.21	100.70	1.66	0.10
	40	2167.00	39.60	99.00	2.36	0.11
Interday	20	1088.65	20.33	101.65	1.46	0.13
	30	1603.00	30.40	101.35	2.00	0.12
	40	2160.03	39.68	99.20	1.80	0.08

**Average of three determination*

Low %RSD values for intra and inter day confirmed that the method is precise.

4.2.2 Intermediate precision

The Intra and Inter-day precision was determined by analysis of the marketed formulation on the same day at different time intervals and on different days respectively. The results are given in Table no. 7.

4.3 Accuracy

To check the accuracy of the proposed method, recovery studies were carried out according to ICH guidelines by applying the standard addition method to known amount, of TA and EMT corresponding to 80,100 and 120%.Analysis was performed as per the procedure given under the tablet analysis. Therecovery studies were performed three times at each level. The results of the recovery studies and its statistical evaluation are summarized in Table no.8 and Table no.9.

Table 8: Results of recovery studies

Recovery Level (%)	**Conc. ofdrug (µg/ml)**		**Total conc. of drug (µg/ml)**	**Total amt. Recovered (µg/ml)**	**% Recovery***	**%RSD**
	Drug Taken	**Std drug added**				
80	10	8	18	17.97	100.02	0.65
100	10	10	20	20.08	100.57	0.49
120	10	12	22	22.14	100.49	1.22

**Average of three determination*

Table 9: Robustness evaluation of TPM

Sr. No.	Factor	Level	Retention time
1	**Flow Rate (ml/min)**		
	0.9	-1	4.50
	1	0	4.35
	1.1	+1	4.05
	Mean± S.D. (n=6)		4.3±0.22
2	**Mobile phase volume (v/v)**		
	89:11	-1	4.50
	90:10	0	4.35
	91:09	+1	4.63
	Mean± S.D. (n=6)		4.50±0.14
3	**Wavelength**		
	262	-1	4.51
	263	0	4.35
	264	+1	4.51
	Mean± S.D. (n=6)		4.45±0.092

Table 10: LOD and LOQ of TPM

Parameter	TPM
LOD(µg/ml)	0.4258
LOQ(µg/ml)	1.2896

4.4 Robustness

The robustness of the method was studied, during development, by small but deliberate variations in flow rate, percentage of methanol in the mobile phase and wavelength. Each factor selected to examine were changed at three levels (-1,0,1)One factor at the time was changed to estimate the effect and to study the effect on the retention time of the drugs.The results are given in Table no.10.

Variation in flow rate, percentage of methanol in the mobile phase and wavelength did not affect the results. Rt and tailing factors of both the drugs at different levels of variations were similar. Hence, the method was found to be robust.

4.5 Limit of Detection (LOD)

The limit of detection (LOD) is the smallest concentration that can be detected but not necessarily quantified as an exact value. LOD is calculated from the formula;

$LOD = 3.3\sigma / S$

Where, σ = standard deviation of the response, S = slope of calibration curve.

4.6 Limit of Quantitation (LOQ)

The limit of quantitation is the lowest amount of analyte in the sample that can be quantitatively determined with precision and accuracy. LOQ is calculated from formula.

$LOQ = 10\sigma / S$

Where, σ = standard deviation of the response, S = slope of calibration curve

LOD, LOQ are shown in the Table no. 11.

Conclusion

From all results it was concluded that the developed RP - HPLC method for the estimation of Topiramate in bulk and pharmaceutical dosage form was accurate, precise, linear, robust, simple and rapid. Percentage recovery shows that the method was free from interference of excipients used in the formulation.

Conflict of Interests

Declared None

Acknowledgement

Authors are grateful to Prof. M. N. Navale, President Sinhgad Technical Education Society for his continuous support and Encore Laboratories Limited, Aurangabad, India for bulk drug gift sample.

Reference

1. Rohit Udhav Bharti*, Vandana Gawande Development and Validation of Spectrophotometric Method for analysis of Topiramate, Asian Journal of Pharmaceutical Technology and Innovation, AJPTI2016, 8-12.
2. Sivakameswari somi reddy*, suresh kannan.v, nagaraju p.t., dr.venugopal. K, development and validation of Spectrophotometric Method for estimation of topiramate bulk and Pharmaceutical dosage form,World Journal of Pharmaceutical Research, 2015,4(9),2272-2280.
3. Sivakameswari somi reddy*, suresh kannan.v, nagaraju p.t., dr.venugopal. K, Analytical method development and validation for estimation of Topiramate bulk and pharmaceutical dosage form by using HPLC method,World Journal of Pharmaceutical Research, 2015,4(10),1043-1060.
4. Viswanath Reddy Pyreddy1*, Useni Reddy Mallu1, Pingili Sunil Reddy2, K. Hussain Reddy1, Maheswara Reddy Musirike1, RP-HPLC/ELSD method determination of topiramate in pharmaceutical products, International Journal of Science Innovations and Discoveries, 2011, 1 (2), 126-133.
5. Ranjana kumara*, Shyamala and 3dr. J. V. C. Sharma, stability indicating method development and validation of topiramate using rp-hplc in bulk and pharmaceutical dosage forms,european journal of pharmaceutical and medical research, 2015,2(7), 264-288.
6. Ali mohammadi, nasrin rezanour, mahdi ansari and roderick b. Walker*, development of a stability-indicating high performance liquid chromatographic method for the analysis of topiramate and dissolution rate testing in topiramate tablets, asian journal of chemistry,2010,vol. 22(5), 3856-3866.
7. ICH Harmonized Tripartite Guideline Validation of Analytical Procedures: Text and Methodology, Q2 (R1) November 2005, 1-17
8. Beckett A. H. and Stenlake J. B., Practical Pharmaceutical Chemistry.4th edition, New Delhi: CBS Publishers and Distributors 2002, 2, 275-288.
9. Sethi P. D., High Performance Liquid Chromatography, Quantitative Analysis of Pharmaceutical Formulations, 1st edition, CBS Publishers and Distributors, New Delhi 2001,3-11, 116-120.

*****