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# RP-HPLC Method Development and Validation For Estimation of Topiramate in Bulk and Tablet Dosage Form

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## ABSTRACT
A simple reverse-phase high performance liquid chromatographic (RP-HPLC) method has been developed and validated for the estimation of Topiramate Chromatography was carried out HPLC water with empower 2 software with isocratic with UV Detector. Selection of column on symmetryC18(4.6X250 mm)and 5um particle size of column , with a mobile phase composed 0f phosphate buffer methanol (35:65 v/v) at a flow rate of mobile was adjusted by 1.0ml/min ,Detection was carried out using a UV detector at 265.nm .Parameters such as linearity, precision, accuracy, ruggedness, LOD and LOQ were studied as per the ICH Q2(R1) guidelines. The retention times were3.744min.for Topiramate The linearity range for Topiramate 0-70µg/ml. The correlation coefficients of Topiramate was found to be 0.999**.** it also done degradation studies to find out found to the loss of drug by acid ,alkali ,thermal, photo, and oxidation and refund within limits. So ,developed method was precise accurate and robust for 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.

## I. INTRODUCTION

Topiramate (brand name Topamax) is an anticonvulsant drug produced by Ortho-McNeil Neurologic, a division of Johnson & Johnson. It is used to treat epilepsy in both children and adults. In children it is also indicated for treatment of Lennox-Gastaut syndrome (a disorder that causes seizures and developmental delays). It is also Food and Drug Administration (FDA) approved for, and now most frequently prescribed for, the prevention of migraines. On August 2013, an extended released formulation, marketed as Trokendi XR has been approved for the management of partial onset, tonic-clonic, and Lennox-Gastaut Syndrome seizures.

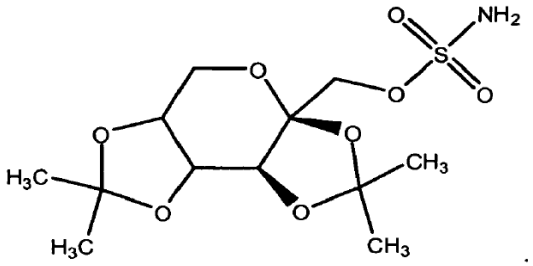

Figure : Structure of Topiramate

The mechanism of action of topiramate is not known. However, studies have shown that topiramate blocks the action potentials elicited repetitively by a sustained depolarization of the neurons in a time-dependent manner, suggesting a state dependent sodium channel blocking action

## II. MATERIAL AND EQUIPMENT

### 2.1 Equipments

- HPLC WATERS with Empower2 Software with Isocratic with UV-Visible Detector,

ELICO SL-159 UV – Vis spectrophotometer,High Precision Electronic Balance Ultra Sonicator (Wensar wuc-2L),Thermal Oven,Symmetry $C_{18}$ Column, 250 mm x 4.6 mm and 5µm particle size**,** $P^H$ Analyzer (ELICO).

### 2.2 Chemicals and Reagents:

HPLC grade water , Methanol, Dipotassium hydrogen orthophosphate, Acetonitrile, Potassium dihydrogen orthophosphate , Ortho phosphoric acid.

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### 2.3 Optimized chromatographic conditions:

Column : Symmetry C18, 250 mm x 4.6 mm i.d.5µm particle size
Mobile Phase : Phosphate Buffer: Methanol (35:65)
Flow Rate: 1.0ml/minute
Wave length: 265 nm
Injection volume: 20 µl
Run time: 7 minutes Column temperature: Ambient

## III. PREPARATION OF MOBILE PHASE AND DILUENT

### 3.1 Preparation of Phosphate buffer:

Weigh exactly 6.8 grams of Potassium dihydrogen orthophosphate and transferred into a 1 litre of beaker, dissolved and diluted up to 1000ml with HPLC Grade water. The pH was adjusted to 5.20 with diluted ortho-phosphoric acid solution.

### 3.2 Mobile phase Preparation:

650 ml of Acetonitrile HPLC (65%) and 350ml (35%) of phosphate buffer are mixed well and degassed in ultrasonic water bath for 15 minutes. The solution was filtered through 0.45µm filter under vacuum filtration

**Mobile phase-A:** Potassium di-hydrogen ortho phosphate buffer solution-(pH5.20)
**Mobile phase-B:** Methanol

### 3.3 Diluent Preparation:

Mobile phase is used as a diluent for the preparation of solutions.

## IV. Preparation of the Topiramate Standard & Sample Solution:

### 4.1 Standard Solution Preparation:

Weigh exactly and transferred 10 mg of Topiramate standard working into a 10ml clean dry volumetric flask add around 8ml of Diluent and sonicated to dissolve it totally and make the volume up to the mark with the same solvent. (Stock solution). From the above stock solution, 0.1ml was pipette into a 10ml volumetric flask and diluted up to the mark with diluent.

### 4.2 Preparation of Sample:

First weigh 20 tablets and estimate the average weight of each tablet. Then the weight which was equivalent to 10mg was transferred into a 500 ml volumetric flask, 260ml of diluent added and sonicated for 25 minutes, further the volume made upto the market with the diluent and filtered. From the filtered solution 1ml was pipeted out into a 10 ml volumetric flask and made upto 10ml with diluent.

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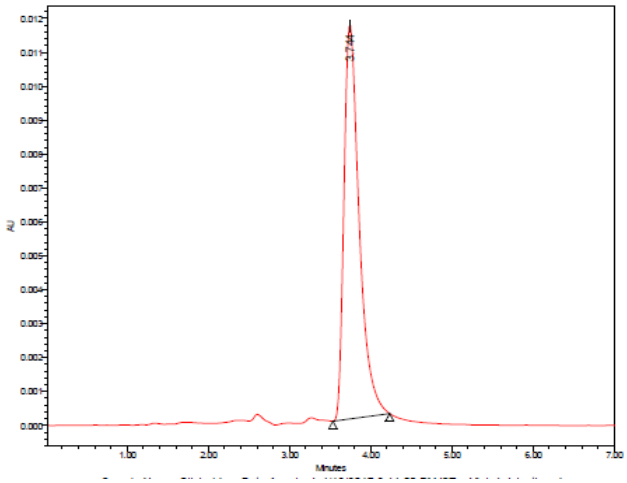

Figure : Estimation of Topiramate in Standard Tablet Dosage Form Standard

| S.No. | Rt | Peak Area | Theoretical Plates | Tailing Factor |
|---|---|---|---|---|
| 1. | 3.744 | 116731 | 3254 | 1.50 |

Table : Results of Topiramate in Standard Tablet Dosage Form Standard

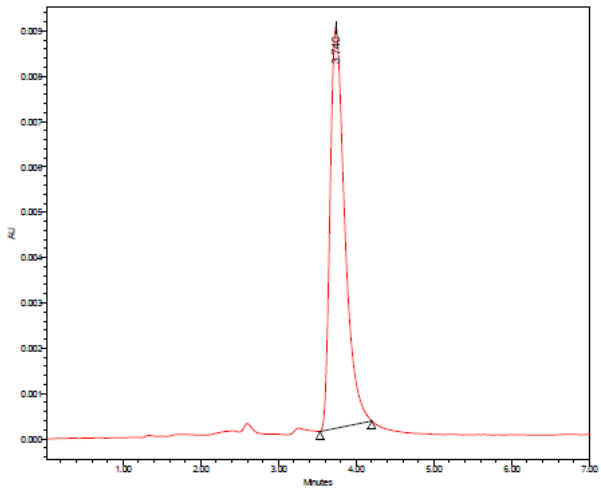

Figure : Results of Topiramate in Standard Tablet Dosage Form Sample

| S.No. | Rt | Peak Area | Theoretical Plates | Tailing Factor |
|---|---|---|---|---|
| 1 | 3.740 | 116731 | 3263 | 1.53 |

Table : Results of Topiramate in Standard Tablet Dosage Form Sample

## V. Validation Parameters :

### 5.1 Accuracy:

The test for accuracy is intended to demonstrate the closeness of agreement between the value found and the value that is accepted either as conventional true value or as an accepted reference value. Thus the accuracy of the method is the closeness of the measured value to the true value for the sample. The accuracy can also be determined by recovery of the impurity spiked to a drug substance or into placebo with drug substance. The percentage recovery with the certain acceptance criteria at each defined level is reported. Accuracy should be assessed using a minimum of nine determinations at a minimum of three concentration levels covering the specified range.

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Table : Accuracy studies of Topiramate tablet dosage form

| ACCURACY 80% | Y | M | C | y-c | X=y-c/m | ADD ppm | %recovery |
|---|---|---|---|---|---|---|---|
| 1 | 90846 | 2663 | 3896 | 86950 | 32.651 | 32 | 102.034 |
| 2 | 89173 | 2663 | 3896 | 85277 | 32.022 | 32 | 100.068 |
| 3 | 88405 | 2663 | 3896 | 84509 | 31.734 | 32 | 99.168 |
| ACCURACY 100% | Y | M | C | y-c | X=y-c/m | ADD ppm | %recovery |
| 1 | 112352 | 2663 | 3896 | 108456 | 40.726 | 40 | 101.815 |
| 2 | 108957 | 2663 | 3896 | 105061 | 39.452 | 40 | 98.63 |
| 3 | 111279 | 2663 | 3896 | 107383 | 40.324 | 40 | 100.81 |
| ACCURACY 120% | Y | M | C | y-c | X=y-c/m | ADD ppm | %recovery |
| 1 | 133541 | 2663 | 3896 | 129645 | 48.683 | 48 | 101.433 |
| 2 | 130283 | 2663 | 3896 | 126387 | 47.460 | 48 | 98.875 |
| 3 | 131456 | 2663 | 3896 | 127560 | 47.900 | 48 | 99.791 |

Table: Accuracy studies for drugs as per % recovery at different levels

| **%Recovery** | **Topiramate** |
|---|---|
| % Recovery at 80% | 102.034 |
| | 100.068 |
| | 99.168 |
| Avg | 100.4233 |
| Standard Deviation | 1.465669 |
| %RSD | 1.45949 |
| % Recovery at 100% | 101.815 |
| | 98.63 |
| | 100.81 |
| Avg | 100.4183 |
| Standard Deviation | 1.628222 |
| %RSD | 1.621439 |
| Recovery at 120% | 101.433 |
| | 98.875 |
| | 99.791 |
| Avg | 100.033 |
| Standard Deviation | 1.296057 |
| %RSD | 1.29563 |

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**5.2 Precision and Reproducibility:**

Precision of a method is the extent to which individual test results of multiple injections of a series of standards agree the measured standard deviation can be sub divided into three categories:

**5.3 Repeatability:**

Repeatability expresses the precision under the same operating conditions over a short interval of time. it is also termed as intra-day precision.

**5.4 Intermediate precision:**

Intermediate precision expresses within laboratories variations: different day, different analyst, different equipments etc

**5.5 Reproducibility:**

Reproducibility expresses the precision between laboratories collaborative studies, usually supplied to standardization of methodology

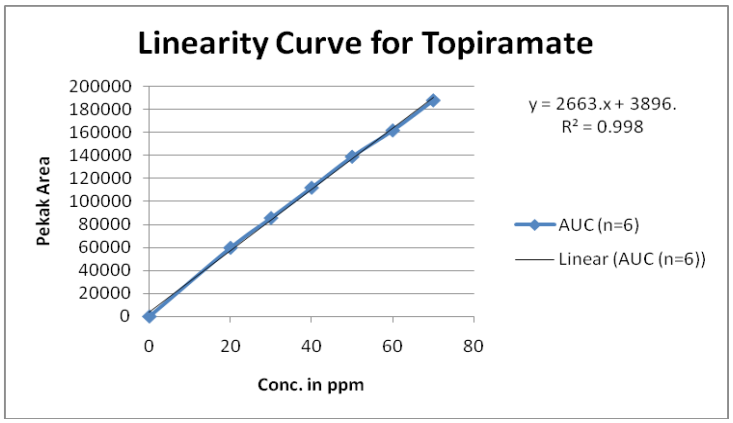
**5.6 Linearity:**

The purpose of the test for linearity is to demonstrate that the entire analytical system (including detector and data acquisition) exhibits a linear response and is directly proportional over the relevant concentration range for the target concentration of the analyte. It is recommended to perform the linearity of the API and related substances independently and once linearity has demonstrated, linearity could be performed containing both API and specific related substance if necessary. At least five concentrations within the range specified above for linearity test should be used.

Table : Linearity results for Topiramate with graph

| S. No. | CONC. (µg/ml) | AUC (n=6) |
|---|---|---|
| 1 | 0 | 0 |
| 2 | 20 | 59731 |
| 3 | 30 | 85677 |
| 4 | 40 | 112028 |
| 5 | 50 | 139014 |
| 6 | 60 | 161889 |
| 7 | 70 | 188021 |

Figure : Linearity Curve for Topiramate




**5.7 Method Robustness:**

To verify the robustness of the method, the analysis was done under variable flow rates, mobile phase & temperature. The flow rate as per the developed method is 1.0 mL/min. This has been purposely changed to 1.2 mL/min and 0.8 mL/min,

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the mobile phase was (65:35) in the ratio of Methanol : Phosphate buffer – purposely changed to decrease and increase of Methanol volume as 67:33 ; 63:37 chromatogram was obtained. By this the developed method was robust no much change to analyse the compound.

Table : Peak area showing the standard at Flow rate of 0.8ml/min – compared with decreased flow rate as 0.6ml/min and increased flow rate as 1.0ml/min.

| S.No. | Standard (1.0ml/ml) | Minus 0.2ml/min | Plus 0.2ml/min |
|---|---|---|---|
| | Topiramate | Topiramate | Topiramate |
| 1 | 116587 | 118547 | 117896 |
| 2 | 117436 | 118321 | 117265 |
| Mean | 117011.5 | 118434 | 117580.5 |
| S.D | 600.3337 | 159.8061 | 446.1844 |
| % RSD | 0.513055 | 0.134933 | 0.379471 |

Table : Peak area showing the standard mobile phase as 65:35 ratio - decrease and increase of Buffer volume as 67:33 ; 63:37.

| S.No. | Standard (65:35) | Minus 0.2ml/min | Plus 0.2ml/min |
|---|---|---|---|
| | Alogliptin | Alogliptin | Alogliptin |
| 1 | 117854 | 118412 | 116985 |
| 2 | 117952 | 118674 | 117484 |
| Mean | 117903 | 118543 | 117234.5 |
| S.D | 69.29646 | 185.262 | 352.8463 |
| % RSD | 0.058774 | 0.156283 | 0.300975 |

**5.8 Limit of detection:** LOD is defined as lowest concentration of the analyte that can be detected, but not necessarily quantified, by the analytical method. **Based on signal to noise ratio**: A signal to noise ratio between 3 or 2:1 is generally considered acceptable for estimating the detection limit.

**5.9 Limit of quantification:** LOQ is defined as the lowest concentration of the analyte that can be determined with acceptable accuracy and precision by the analytical method. **Based on signal to noise ratio**: signal to noise ratio between 10:1 is generally constant

Table: Showing LOD and LOQ for the drugs.

| | Topiramate |
|---|---|
| LOD | 0.045682 |
| LOQ | 0.125684 |

## 6. STABILITY STUDIES

**Results of degradation studies:**

The results of the stress studies indicated the specificity of the method that has been developed. Topiramate was stable in acidic and thermal stress conditions. The result of forced degradation studies are given in the following table.

Table : Degradation studies for Alogliptin

| Solvents | Alogliptin | |
|---|---|---|
| | Peak area | % Drug |
| Mobile phase<br>Methanol: Phosphate Buffer (65:35) | 116731 | 100.00 |
| Acidic – 0.1N – HCl | 116708 | 99.98 |
| Alkaline – 0.1N – NaOH | 114502 | 98.09 |

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| | | |
|---|---|---|
| Thermal - 150°C - 24hrs. | 116719 | 99.99 |
| Photolytic - UV-light. | 114012 | 97.68 |
| Oxidation - 20% - $H_2O_2$ | 99957 | 85.63 |

## VI. CONCLUSION

A simple reverse phase HPLC method was developed for the estimation of Topiramate in bulk and tablet dosage form. A Symmetry C18, 250 mm x 4.6 mm i.d.5µm particle size column from Waters in isocratic mode, with mobile phase containing Phosphate Buffer: Methanol (35:65). The flow rate was 1.0ml/min and effluent was monitored at UV wavelength of 265.0 nm. The retention times were 3.744 min for Topiramate. The linearity and range was found to be in the range of 0-70 µg/ml for Topiramate. The correlation coefficient of Topiramate was found to be 0.999, which indicates a perfect correlation. The method was validated as per ICH guidelines for accuracy calculated as % recovery was in the range of 98.0% to 102.0%. Accuracy, precision, system suitability, LOD & LOQ were determined. Degradation studies were done to find the loss of drug by acid, alkali, thermal, photo & oxidation, all are found within the limit so it was concluded that the developed method was precise, accurate and robust for determination of % purity in formulation of combined tablet dosage form. The statistical analysis of the data showed that the method is reproducible and selective for the estimation of Topiramate in tablet dosage form during routine analysis.

## BIBILOGRAPHY

[1]. R. Patil: J of Chromatographia, 67, 575,(2008).
[2]. Rasayan Journal of chemistry Vol. 5 | No.1 | 90-105 | January-March | 2012ISSN: 0974-1496 | CODEN: RJCABP.
[3]. IUBMB Life. 2009 Apr;61(4):470-5. Doi: 10.1002/iub.181.
[4]. Baht and Leena:J of Liq. Chrom., 30, 309, (2007).
[5]. H.H.Williard, L.L.Merit, F.A.Dean and F.A.Settle, Instrumental methods of analysis, 7th edition, C.B.S.Publishers, New Delhi,( 2002).
[6]. GN Menon, LB White, Department of Analytical Research,Abbott Laboratories, (pubmed- index for MEDLINE).
[7]. International Conference on Harmonization, "Q2A: Text on Validation of Analytical Procedures," Federal Register 60(40), 11260–11262 (1995).
[8]. International Conference on Harmonization, "Q2B: Validation of Analytical Procedures: Methodology; Availability," Federal Register 62(96), 27463–27467 (1997).
[9]. FDA, "Analytical Procedures and Methods Validation: Chemistry, Manufacturing and Controls Documentation; Availability," Federal Register (Notices) 65(169), 52776–52777 (2000).
[10]. G.A. Shabir, "Validation of HPLC Chromatography Methods for Pharmaceutical Analysis. Understanding the Differences and Similarities Between Validation Requirements of FDA, the US Pharmacopeia and the ICH," J. Chromatogr. A. 987(1-2), 57-66 (2003).
[11]. N. Torrealday: J of Pharmaceutical and biomed. Ana., 32, 847, (2003).
[12]. Validation of analytical procedures, Methodology, ICH harmonized tripartite guideline, 108, 1996.
[13]. FDA Drug Approvals List [online](cited 26 Aug 2003).
[14]. J. M. Green, A practical guide to analytical method validation, Anal. Chem. News & Features, 1 May 1996, pp. 305A–309A.
[15]. P. A. Winslow and R. F. Meyer, Defining a master plan for the validation of analytical methods, J. Validation Technology, pp. 361–367 (1997).
[16]. Mahadev B Kshirsagar*, Moreshwar P. Mahajan, Sanjay D. Sawant., Method Development and Validation by RP-HPLC for Estimation of Topiramate in Bulk and Pharmaceutical Dosage form, International Journal of ChemTech Research, Vol.10 No.7, pp 843-849, 2017.
[17]. 1*Ranjana Kumari, 2 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, ejpmr, 2015,2(7), 264-288.
[18]. Sivakameswari Somireddy*, Sureshkannan.V, Nagaraju P.T., Dr.Venugopal. K, Muralikrishna. N.V,

International Journal of Pharmaceutical research and Applications
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Ramadevi.R, analytical method development and validation for estimation of topiramate bulk and pharmaceutical dosage form by using hplc method, World Journal of Pharmacy and Pharmaceutical Sciences, Volume 4, Issue 10, 1043-1060.

[19]. 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 Vol. 22, No. 5 (2010), 3856-3866.

[20]. 1*Ranjana Kumari, 2 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, ejpmr, 2015,2(7), 264-288.

[21]. Sivakameswari Somireddy*, Sureshkannan.V, Nagaraju P.T., Dr.Venugopal. K, Muralikrishna. N.V, Ramadevi.R, analytical method development and validation for estimation of topiramate bulk and pharmaceutical dosage form by using hplc method, World Journal of Pharmacy and Pharmaceutical Sciences, Volume 4, Issue 10, 1043-1060.

[22]. 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 Vol. 22, No. 5 (2010), 3856-3866.

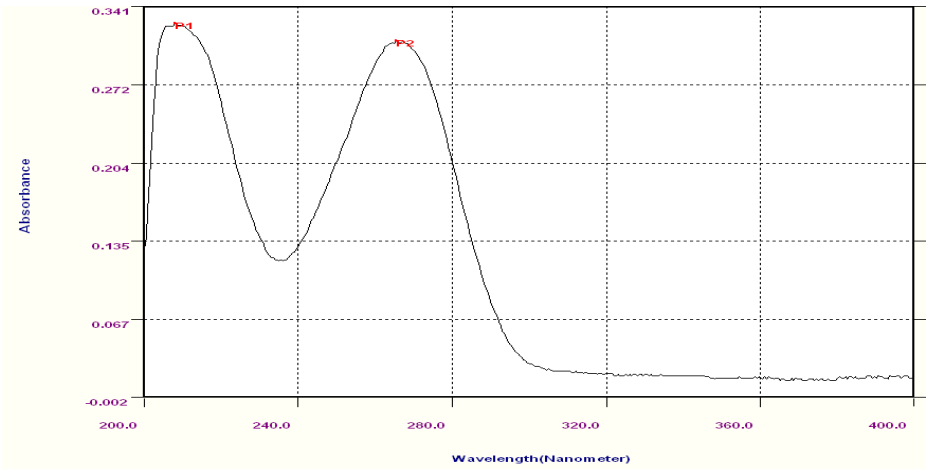
## METHOD DEVELOPMENT AND OPTIMIZATION

To undertake solubility and analytical studies of Topiramate and to develop initial UVand chromatographic conditions.. Setting up of initial UV and chromatographic conditions for the method development in pure and pharmaceutical dosage forms. Optimization of initial chromatographic and spectrophotometric conditions.. Analytical method validation of the developed RP- HPLC method. Quantitative determination of Topiramate in pharmaceutical dosage form using the method developed and validated.

**Sample and Standard preparation for the UV-spectrophotometer analysis**:

10mg of Topiramate standard was transferred into 10ml volumetric flask, dissolved & make up to volume with mobile phase. Additionally dilution was done by transferring 1ml of the resulted above solution into a 10ml volumetric flask and make up the volume up to the mark with the mobile phase

The standard & sample stock solutions were prepared separately by dissolving standard & sample in a solvent in mobile phase diluting with the same solvent.(After optimization of all conditions) for UV analysis. It scanned in the UV spectrum in the range of 200 to 400nm. While scanning the Topiramate solution we observed the maxima at 265 nm. The UV spectrum was recorded on ELICO SL-159 make UV-Vis spectrophotometer model UV-2450. The UV spectrum scanned is attached in



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**Mobile Phase:**
**Mobile phase preparation:**
The mobile phase used in this analysis consists of a mixture of Phosphate Buffer
(0.01M potassium dihydrogen phosphate & pH adjusted to 5.2 with ortho phosphoric acid)
and Methanol in a ratio of 35:2
**Preparation of Standard solution:**
Working concentration should be around 40µg/ml.
Accurately weighed around 25mg of Topiramate working standard, taken into a 25 ml
volumetric flask, then dissolved and diluted to volume with the mobile phase to obtain a
solution having a known concentration of about 1000 mcg/ml.Further dilutions has been made to get the final concentration of 40 µg/ml
**Preparation of Test solution:**
Diluted quantitatively an accurately measured volume of label claim solution with diluents to
obtain a solution containing about a linear range.

**PREPARATION OF MOBILE PHASE AND DILUENT:**
**1. Preparation of Phosphate buffer:**
Weigh exactly 6.8 grams of Potassium dihydrogen orthophosphate andtransferred into a 1 litre of beaker, dissolved and diluted up to 1000ml with HPLC Gradewater. The pH was adjusted to 5.20 with diluted ortho-phosphoric acid solution.
**2. Mobile phase Preparation**:
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and degassed in ultrasonic water bath for 15 minutes. The solution was filtered through
0.45µm filter under vacuum filtration.
**Mobile phase-A :**Potassium di-hydrogen ortho phosphate buffer solution-(pH 5.20)
**Mobile phase-B :**Methanol
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Mobile phase is used as a diluent for the preparation of solutions.
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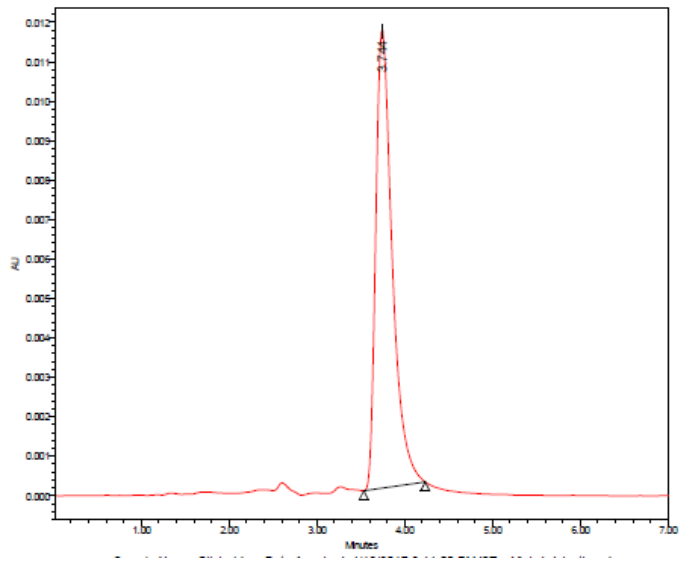


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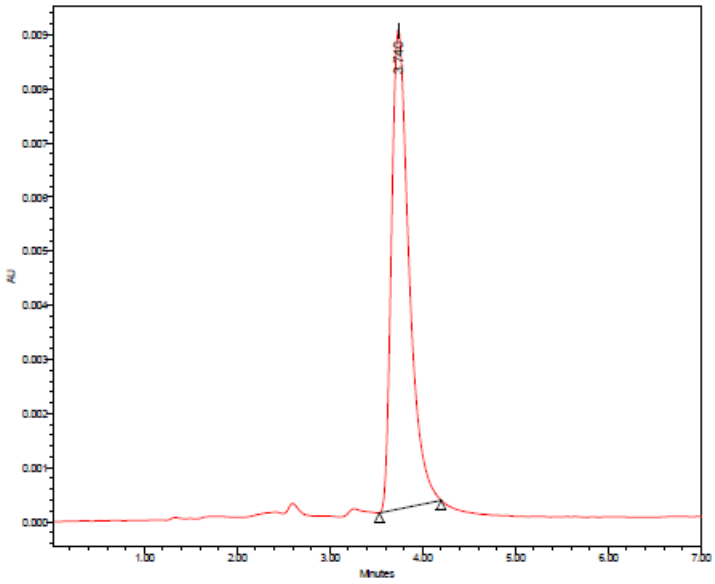


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METHOD VALIDATION

Accuracy**:**

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| % Recovery | Topiramate |
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| Avg. | **100.4233** |
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| | 99.791 |
| Avg. | **100.033** |
| Standard Deviation | **1.296057** |
| %RSD | **1.29563** |

Table: Accuracy studies for drugs as per % recovery at different levels

**Precision and Reproducibility:**

Precision of a method is the extent to which individual test results of multiple injections of a series of standards agree the measured standard deviation can be sub divided into three categories:

**Repeatability:** Repeatability expresses the precision under the same operating conditions over a short interval of time. it is also termed as intra-day precision.

**Intermediate precision:** intermediate precision expresses within laboratories variations: different day, different analyst, different equipmentsetc

**Reproducibility:** Reproducibility expresses the precision between laboratories collaborative studies, usually supplied to standardization of methodology.

**Linearity:**

The purpose of the test for linearity is to demonstrate that the entire analytical system (including detector and data acquisition) exhibits a linear response and is directly proportional over the relevant concentration range for the target concentration of the analyte. It is recommended to perform the linearity of the API and related substances independently and once linearity has demonstrated, linearity could be performed containing both API and specific related substance if necessary. At least five concentrations within the range specified above for linearity test should be used.

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Table : Linearity results for Topiramate with graph.

| S. No. | CONC. (µg/ml) | AUC (n=6) |
|---|---|---|
| 1 | 0 | 0 |
| 2 | 20 | 59731 |
| 3 | 30 | 85677 |
| 4 | 40 | 112028 |
| 5 | 50 | 139014 |
| 6 | 60 | 161889 |
| 7 | 70 | 188021 |

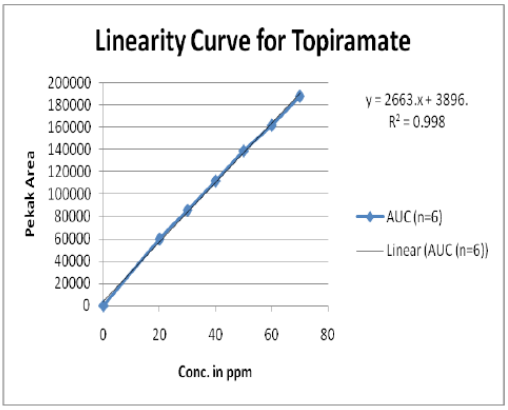


Figure: Linearity Curve for Topiramate

**(iv) Limit of Detection:**

LOD is defined as lowest concentration of the analyte that can be detected, but not necessarily quantified, by the analytical method.

The limit of detection (LOD) may be expressed as LOD = 10 /S

Where: = the standard deviation of the response; S = slope of calibration curve of analyte.

**(v) Limit of Quantification:**

LOQ is defined as the lowest concentration of the analyte that can be determined with acceptable accuracy and precision by the analytical method.

The limit of detection (LOQ) may be expressed as LOQ = 10 /S

Where: = the standard deviation of the response; S = slope of calibration curve of analyte.

| | Topiramate |
|---|---|
| LOD | 0.045682 |
| LOQ | 0.125684 |

Table: Showing LOD and LOQ for the drugs.

**(vi) Robustness:**

Robustness tests examine the effect operational parameters have on the analysis results by changing the Flow rate, mobile phase ratio, column temperature, change of column, etc;

**6.5. Degradation studies:**

**(i) Acid Degradation Studies:**

To 1ml of stock solution Topiramate, add 1ml of 0.1N- HCl (Hydrochloric acid) was added and kept for 24hrs.The resultant solution was diluted to obtain 10µg/ml and 10µg/ml solutions and 10µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

**(ii) Alkali Degradation Studies:**

To 1 ml of stock solution Topiramate, 1 ml of 1N sodium hydroxide was added and kept for 24hrs. The resultant solution was diluted to obtain 10µg/ml and 10µg/ml solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

**(iii) Dry Heat Degradation Studies:**

The standard drug solution was placed in oven at 500c for 24hrs to study dry heat degradation. For HPLC study, the resultant solution was diluted to 10µg/ml and 10µg/ml solutions and 10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample**.**

**(iv)Photo Stability studies:**

The photochemical stability of the drug was also considered by revealing the 10µg/ml and 10µg/ml solution to UV Light (254nm) by maintenance the beaker in UV Chamber for 24hrs, Page 32

For HPLC study the ensuing solution was diluted to get hold of 10µg/ml and 10µg/ml solutions and 10 µl were injected into the system and the chromatograms were record to review the stability of sample.

**(v) Oxidation Stability Studies:**

To 1 ml of stock solution of Topiramate, 30ml of 3% hydrogen peroxide (H2O2) was added separately. The solutions were kept for 24hrs. For HPLC study, the resultant solution was diluted to obtain 10µg/ml and 10µg/ml solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

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| Solvents | Peak area | % Drug |
|---|---|---|
| Mobile phase | 1161131 | 100.00 |
| Methanol: Phosphate Buffer (65:35) | | |
| Acidic – 0.1N – HCl | 1167108 | 99.98 |
| Alkaline – 0.1N – NaOH | 1142205 | 98.09 |
| Thermal - 150°C - 5hrs | 1165119 | 99.99 |
| Photolytic - UV-light | 1140113 | 97.98 |
| Oxidation - 30% - $H_2O_2$ | 999521 | 85.93 |

Table : Degradation studies for Alogliptin

## 7. CONCLUSION

A simple reverse phase HPLC method was developed for the estimation of Topiramate in bulk and tablet dosage form. A Symmetry C18, 250 mm x 4.6 mm i.d.5µm particle size column from Waters in isocratic mode, with mobile phase containing Phosphate Buffer: Methanol (35:65). The flow rate was 1.0ml/min and effluent was monitored at UV wavelength of 265.0 nm. The retention times were 3.744 min for Topiramate. The linearity and range was found to be in the range of 0-70 µg/ml for Topiramate. The correlation coefficient of Topiramate was found to be 0.999, which indicates a perfect correlation. The method was validated as per ICH guidelines for accuracy calculated as % recovery was in the range of 98.0% to 102.0%. Accuracy, precision, system suitability, LOD & LOQ were determined. Degradation studies were done to find the loss of drug by acid, alkali, thermal, photo& oxidation, all are found within the limit so it was concluded that the developed method was precise, accurate and robust for determination of % purity in formulation of combined tablet dosage form. The statistical analysis of the data showed that the method is reproducible and selective for the estimation of Topiramate in tablet dosage form during routine analysis.

## BIBLIOGRAPHY

[1]. R. Patil: J of Chromatographia, 67, 575,(2008).
[2]. Rasayan Journal of chemistry Vol. 5 | No.1 | 90-105 | January-March | 2012ISSN: 0974-1496| CODEN: RJCABP.
[3]. IUBMB Life. 2009 Apr;61(4):470-5. Doi: 10.1002/iub.181.
[4]. Baht and Leena:J of Liq. Chrom., 30, 309, (2007).
[5]. H.H.Williard, L.L.Merit, F.A.Dean and F.A.Settle, Instrumental methods of analysis, 7th
[6]. edition, C.B.S.Publishers, New Delhi,( 2002).
[7]. GN Menon, LB White, Department of Analytical Research,Abbott Laboratories, (pubmedindexfor MEDLINE).
[8]. International Conference on Harmonization, "Q2A: Text on Validation of Analytical
[9]. Procedures," Federal Register 60(40), 11260–11262 (1995).
[10]. International Conference on Harmonization, "Q2B: Validation of Analytical Procedures:
[11]. Methodology; Availability," Federal Register 62(96), 27463–27467 (1997).
[12]. FDA, "Analytical Procedures and Methods Validation: Chemistry, Manufacturing and
[13]. Controls Documentation; Availability," Federal Register (Notices) 65(169), 52776–52777
[14]. (2000).
[15]. G.A. Shabir, "Validation of HPLC Chromatography Methods for Pharmaceutical Analysis.Understanding the Differences and Similarities Between Validation Requirements of FDA,the US Pharmacopeia and the ICH," J. Chromatogr. A. 987(1-2), 57-66 (2003).
[16]. N. Torrealday: J of Pharmaceutical and biomed. Ana., 32, 847, (2003).
[17]. Validation of analytical procedures, Methodology, ICH harmonized tripartite guideline, 108,1996.
[18]. FDA Drug Approvals List [online](cited 26 Aug 2003).
[19]. J. M. Green, A practical guide to analytical method validation, Anal. Chem. News &
[20]. Features, 1 May 1996, pp. 305A–309A.
[21]. P. A. Winslow and R. F. Meyer, Defining a master plan for the validation of analytical
[22]. methods, J. Validation Technology, pp. 361–367 (1997).
[23]. Mahadev B Kshirsagar*, Moreshwar P. Mahajan, Sanjay D. Sawant., Method Development and Validation by RP-HPLC for Estimation of Topiramate in Bulk and Pharmaceutical Dosage form, International Journal of ChemTech Research, Vol.10 No.7, pp 843-849, 2017.
[24]. 1*RanjanaKumari, 2 Shyamala and 3Dr. J. V. C. Sharma, stability indicating method
[25]. development and validation of topiramate using rp-hplc in bulk and pharmaceutical dosage

[26]. forms, european journal of pharmaceutical and medical research, ejpmr, 2015,2(7), 264-288.
[27]. SivakameswariSomireddy*, Sureshkannan.V, Nagaraju P.T., Dr.Venugopal. K,
[28]. Muralikrishna. N.V, Ramadevi.R, analytical method development and validation for
[29]. estimation of topiramate bulk and pharmaceutical dosage form by using hplc method, World
[30]. Journal of Pharmacy and Pharmaceutical Sciences, Volume 4, Issue 10, 1043-1060.
[31]. Ali mohammadi, nasrinrezanour, mahdiansari and roderick b. walker*, Development of a Stability-Indicating High Performance Liquid Chromatographic Method for the Analysis of
[32]. Topiramate and Dissolution Rate Testing in Topiramate Tablets, Asian Journal of Chemistry
[33]. Vol. 22, No. 5 (2010), 3856-3866.