ejpmr, 2015,2(7), 264-288

SJIF Impact Factor 2.026

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 3294-3211

EJPMR

STABILITY INDICATING METHOD DEVELOPMENT AND VALIDATION OF TOPIRAMATE USING RP-HPLC IN BULK AND PHARMACEUTICAL DOSAGE FORMS

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Article Received on 11/10/2015 **Article Revised on 03/11/2015** **Article Accepted on 26/11/2015**

ABSTRACT

The present project work is aimed on stability indicating method development and validation of topiramate using RP-HPLC in bulk and pharmaceutical dosage form . The method developed will be accurate, precise and linear for the estimation of the drugs in the near future. The analytical method will be developed by studying different parameters of Topiramate. The column used for study is Inertsil C18, ODS chosen good peak shape. Ambient temperature is found to be suitable for the nature of drug solution. The flow rate will be fixed at 1.0ml/min to obtain good peak area, satisfactory retention time and good resolution. Different ratios of mobile phase will be studied, mobile phase with satisfactory results will be selected for good symmetrical peaks and good resolution. We conduct the analysis based on UV detection and Reverse Phase High Performance Liquid Chromatograph.

KEYWORDS: Topiramate, RP-HPLC, UV spectrometer.

INTRODCUTION

Pharmaceutical Analysis plays a very vital role in maintaining the quality control and assurance ample drugs and their formulations. Pharmaceutical analysis is a special branch of chemistry analysis which dealswith identifying, separating and determining the qualified amounts of compounds in a trial of matter. It is concerned with the chemical characterization of matter both quantitative and qualitative.

In recent years, several analytical techniques have been evolved.

SPECTROPHOTOMETRIC METHODS

Spectrophotometry is generally considered by the small scale industries as the equipments cost are minimal and also its maintenance reduces. The method of analysis is done by measuring the absorbed monochromatic light by the ultra violet path of spectrum (200-380nm). The photometric analysis is constructed using the Bouger – Lamberts –Beer Law which state that solution absorbance is directly proportional to the analytic concentration. The fundamental principle of the spectrophotometer operation is to cover the UV region consistently in the light which has a definite wavelength intervals which passes through a cell solvent that falls on the photoelectric cell that converts the radiant energy into electric energy which is in turn measured using a galvanometer.

The important applications are

1. Identification of many types of organic, inorganic molecules and ions.
2. Quantitative determination of many biological, organic and inorganic species.
3. Scrutinizing and identification of chromatographic of effluents.

HPLC METHOD DEVELOPMENT

The term ‘Chromatography’is termed as a process which focuses on the separation of various classes of mixture depending on their classification of characteristics between distinct and discrete phases

1.2.1MODES OF CHROMATOGRAPHY

Mode of chromatography emphasizing on the nature of interactions between solute and distinct phases which is developed from hydrodgen bond, vander walls force etc.,

Various modes of chromatography are as follows

1. Normal Phase Chromatography
2. Reversed Phase Chromatography

3. Reversed Phase – ion pair Chromatography
4. Ion-Exchange Chromatography
5. Size Exclusion Chromatography

REVERSED PHASE CHROMATOGRAPHY

The objective was to make less polar or non polar so that polar solvents can be used to separate water-soluble polar compounds. Since the ionic nature of the chemically modified silica is now reversed i.e. it is non-polar or the nature of the phase is reversed. The chromatographic separation carried out with such silica is referred to as reversed- phase chromatography.

A large number of chemically bonded stationary phases based on silica are available commercially. Silica based stationary phases are still most popular in reversed phase chromatography however other adsorbents based on polymer (styrene-divinyl benzene copolymer) are slowly gaining ground.

Simple compounds are retained by the reversed surface phase , which are less water-soluble .the retention decreases in following order:
aliphatics> induced dipoles (i.e. CCl_4) > permanent dipoles (e.g.$CHCl_3$) > weak Lewis bases (ethers, aldehydes, ketones) > strong Lewis bases (amines) > weak Lewis acids (alcohols, phenols) > strong Lewis acids (carboxylic acids).

Also the retention increases as there is an increase in the number of carbons.

The general rule of the retention increases with an increase in contact area between sample molecule and stationary phases which are emitted during the comound absorption. In comparision freed with the normal isomers, the branched chain compound elute more rapidly

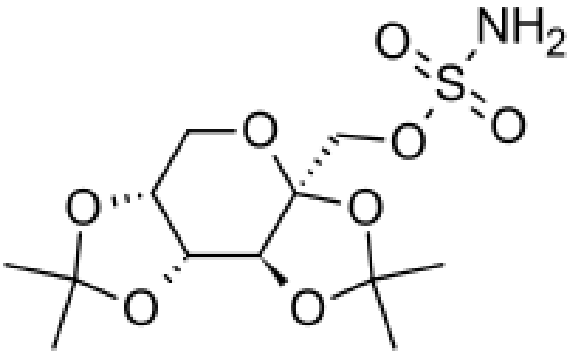
In the pharmaceuticals industries the chemically bound octadecyl silane (ODS) with 18 carbonare most popular stationary phases utilized. As most of the compounds are polar and water solution which are used maximum in HPLC methods which determines quality control, assurance, analysis of ample drugs and its formulation. The solvent strength is reversed from the chromatography absorption, as water interacts with silanol groups, so that the sample becomes restricted and elutes rapidly.

The exact reverse is applied when the water cannot wet the non polar ie., alkyl groups, which doesn't interacts with mositly bound compound , hence water is the weakest solvent with slow elution. The retention time increases with the increase in amount of water in mobile phase.

DRUG PROFILE

1. TOPIRAMATE

Structure



IUPAC :2,3:4,5-Bis-O-(1-methylethylidene)-beta-D-fructopyranose sulfamate

Chemical formula : $C_{12}H_{21}NO_8S$

Molecular Weight : 339.363 g/mol

Solubility : Soluble in water (9.8 mg/ml)

Soluble in most alkaline solutions containing sodium hydroxide or sodium phosphate and having pH of 9 - 10. Freelysoluble in acetone, chloroform, dimethyl, sulfoxide and ethnol.

Mechanism of Action: The precise 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. 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 to its anticonvulsant actions.

Half life: 19 to 23 hours. The mean elimination half-life was 31 hours following repeat administration of the extended-release formulation.

Brand Names

a. Topamax
b. Trokendi XR
c. Topiragen
d. Qudexy XR

Research Methodologies: In this paper we conduct the analysis using the below methods:

METHOD DEVELOPMENT FOR HPLC

HPLC EQUIPMENTS

S.No	Name	Model
1.	HPLC Instrument series software	PROMINANCE SHIMADZU- SPD20A
2.	Column	INERTSIL ODS (250mm,4.6mm,5µ) HYPERSIL C_{18} (100,4.6mm,5 µ)
3.	Detector	UV-Visible detector

5.1.2 OTHER EQUPIMENTS

S.No	Name	Model
1.	Analytical Balance	SHIMADZU
2.	Sonicator	ULTRA SONICA CLEANER
3.	pH Meter	Globel Digital pH Meter
4.	Vacuum Filter	Model XI 5522050o Millipore

5.1.3 REAGENTS USED FOR THE STUDY

S.No	Reagent	Grade	Molecular Formula
1.	Potassium dihydrogen orthophosphate	Merck – HPLC Grade	KH_2PO_4
2.	Aceteonitrile	HPLC Grade	CH_3CN
3.	Methanol	Standard Reagent pvt	CH_3OH
4.	Water	Milli Q Grade	H_2O
5.	Sodium Hydroxide		NaOH
6.	Hydrochloric Acid		HCl

5.1.4 SAMPLE

S.No	Working Standard	Potency
1.	Topiramate	98.8 %

5.1.5 METHOD DEVELOPMENT

The objective of this experiment was to optimize the assay method for estimation of Topiramate based on the literature survey made. So here the trials mentioned describes how the optimization was done

Trial: 1

Mobile Phase: 100% pure degaussed methanol.

Preparation of Standard Solution

10mg of f Topiramate drug was weighed and dissolved in 10ml of Mobile phase and taken in 10ml of volumetric flask and sonicated for 20 minutes to get 1000ppm and 1 ml was taken from this and diluted to 10 ml with mobile phase

Chromatographic Conditions

Flow rate : 1.0ml/min
Column : Inertsil - C18 ODS column
Detector wavelength : 263 nm
Column temp : Ambient
Injection volume : 20µl
Run time : 10min
Retention time : 4.428

Observation: Theoretical plates are less, peak shape is not good and asymmetry is more than limit. . The trial 1 chromatogram result was shown in Fig:1

Trail: 2.

Mobile Phase: methanol and Acetonitrile were mixed in the ratio of 90:10V/V and sonicated to degas.

Preparation of Standard Solution

10mg of Topiramate drug was weighed and dissolved in 10ml of Mobile phase and taken in 10ml of volumetric flask and sonicated for 20 minutes to get 1000ppm and 1 ml. was taken from this and diluted to 10ml.with mobile phase

Chromatographic Conditions

Flow rate : 1ml/min
Column : Inertsil -C18 ODS column
Detector wavelength : 263 nm
Column temp : Ambient
Injection volume : 20µl
Run time : 10min
Retention time : 2.418

Observation: Got Base Line Noice, peak tailing occurs. The trial 2 chromatogram result was shown in Fig:

Trail: 3.

Mobile Phase: Methanol and Acetonitrile were mixed in the ratio of 80:20 V/V and sonicated to degas.

Preparation of Standard Solution:
10mg of Topiramate drug was weighed and dissolved in 10ml of Mobile phase and taken in 10ml of volumetric flask and sonicated for 20 minutes to get 1000ppm and 1 ml. was taken from this and diluted to 10ml.with mobile phase

Chromatographic Conditions:
Flow rate : 1.0ml/min
Column : Inertsil - C18 ODS column
Detector wavelength : 263 nm
Column temp : Ambient
Injection volume : 20µl
Run time : 10min
Retention time : 1.815

Observation: Got lesss retenton time and peak is spleated. The trial 3chromatogram result was shown in Fig:3

5.2 OPTIMIZED METHOD
Mobile Phase: acetonitrile and Methanol were taken and sonicated to degas in the ratio of 70:30

Preparation of stock solution: 10mg of Topiramate drug was weighed and dissolved in 10ml of Mobile phase and taken in 10ml of volumetric flask and sonicated for 20 minutes to get 1000ppm and 2 ml. was taken from this and diluted to 10ml.with mobile phase.

Preparation of working standard solution
The stock solution equivalent to 20ppm to 80ppm were prepared, sonicated and filtered through 0.45µ membrane.

Preparation of sample drug solution for pharmaceutical formulations
Twenty tablets containing Topiramate of each marketed formulation were taken and powdered. The powder equivalent to 10 mg of the active ingredient was accurately weighed and taken in a 10ml volumetric flask containing 50 ml mobile phase and sonicated for 15 minutes and the solution was made up to volume with mobile phase and filtered through 0.45micron membrane.

Procedure for calibration curve
The contents of the mobile phase were filtered before use through 0.45micron membrane and pumped from the respective solvent reservoirs to the column at a specified flow rate. Prior to injection of the drug solutions, the column was equilibrated for at least 30min with the mobile phase flowing through the system. The chromatographic separation was achieved using a mobile phase consisting of Acetonitrile at 100v/v the eluent was monitored using UV detector at a wavelength of 263 nm. The column was maintained an ambient temperature (25^0c) and an injection volume of 10µl of each of standard and sample solutions were injected into the HPLC system to get the chromatograms. The retention time, peak area of drug was recorded graph was plotted by taking concentration of the drug on x-axis and peak area on y-axis. A typical chromatogram of Topiramate was shown in Fig 4.

Optimized chromatographic conditions

PARAMETERS	***METHOD***
Stationary phase (column)	Inertsil -ODS C_{18} (250 x 4.6 mm, packed with 5 micron)
Mobile Phase	Acetonitrile:Methanol(70: 30)
Flow rate (ml/min)	1.0 ml
Run time (minutes)	10
Column temperature (°C)	Ambient
Volume of injection loop (µl)	20
Detection wavelength (nm)	263 nm
Drug RT(min)	2.345

CALCULATION
The amount of drug present in each pharmaceutical formulation was calculated by using the standard calibration curves (concentration in ppm was taken on x-axis and peak area on y-axis). A typical chromatogram of Topiramate (100ppm) (formulation) was shown in Fig: 5

5.3 METHOD VALIDATION
5.3.1SYSTEM SUITABILITY
A Standard solution was prepared by using Topiramate working standard as per test method and was injected Five times into the HPLC system.

The system suitability parameters were evaluated from standard chromatograms by calculating the % RSD from five replicate injections for Topiramate**,** retention times and peak areas.

ACCEPTANCE CRITERIA
1. The % RSD for the retention times of principal peak from 5 replicate injections of each Standard solution should be not more than 2.0 %
2. The % RSD for the peak area responses of principal peak from 5 replicate injections of each standard Solution should be not more than 2.0%.
3. The number of theoretical plates (N) for the Topiramate peaks is NLT 3000.
4. The Tailing factor (T) for the Topiramate peaks is NMT 2.0

OBSERVATION
The %RSD for retention times and peak areas were found to be within the limit.refer table: 1 As sown in fig 6 – 10.

5.3.2 SPECIFICITY
Topiramate identification
Solutions of standard and sample were prepared as per the test method are injected into chromatographic system.

ACCEPTENCE CRITERIA
Chromatogram of standard and sample should be identical with near Retention time.

OBSERVATION
The chromatograms of Standard and Sample were same identical with same retention time. As shown in fig: 12 and fig: 13.

5.3.3 PRECISION
5.3.3.1Repeatability
a. System precision: Standard solution prepared as per test method and injected five times.
b. Method precision: Prepared six sample preparations individually using single as per test method and injected each solution.

ACCEPTANCE CRITERIA: The % relative standard deviation of individual Topiramate , from the six units should be not more than 2.0%.
The assay of Topiramate should be not less than 98% and not more than 102.0%.

OBSERVATION
Test results are showing that the test method is precise. Refer tables 2 and 3 for system precision and for method precision.

5.3.3.2Intermediate precision (analyst to analyst variability)
A study was conducted by two analysts as per test method

ACCEPTENCE CRITERIA
The individual assays of Topiramate should be not less than 98% and not more than 102% and %RSD of assay should be NMT2.0% by both analysts.

OBSERVATION
Individual %assays and %RSD of Assay are within limit and passes the intermediate precision, Refer table: 4

5.3.4 ACCURACY (RECOVERY)
A study of Accuracy was conducted. Drug Assay was performed in triplicate as per test method with equivalent amount of F Topiramate into each volumetric flask for each spike level to get the concentration of f Topiramate equivalent to 50%, 100%, and 150% of the labeled amount as per the test method. The average % recovery of Topiramate was calculated.

ACCEPTANCE CRITERIA
The mean % recovery of the Topiramate at each spike level should be not less than 98.0% and not more than 102.0%.

OBSERVATION

$$\%\text{Recovery} = \frac{\text{Amount found}}{\text{Amount added}} \times 100$$

The recovery results indicating that the test method has an acceptable level of accuracy. Refer table: 5

5.3.5LINEARITY OF TEST METHOD
A Series of solutions are prepared using Topiramate working standard at concentration levels from 20ppm to 80 ppm of target concentration .Measure the peak area response of solution at Level 1 and Level 6 six times and Level 2 to Level 5 two times.

ACCEPTANCE CRITERIA
Correlation Coefficient should be not less than 0.9990.
% of y- Intercept should be ±2.0.
% of RSD for level 1 and Level 6 should be not more than 2.0%.

OBSERVATION
The linear fit of the system was illustrated graphically. The results are presented in table6.

5.3.6 RUGGEDNESS OF TEST METHOD
a) System to system variability
System to system variability study was conducted on different HPLC systems, under similar conditions at different times. Six samples were prepared and each was analyzed as per test method.

Comparison of both the results obtained on two different HPLC systems, shows that the assay test method are rugged for System to system variability.

ACCEPTANCE CRITERIA
The % relative standard deviation of Topiramate from the six sample preparations should be not more than 2.0%
The % assay of Topiramate should be between 98.0%-102.0%.

OBSERVATION
The % RSD was found within the limit. Ref tables: 3 &7.

b) column to column variability
Column to column variability study was conducted by using different columns. Six samples were prepared and each was analysed as per test method

ACCEPTANCE CRITERIA
The% RSD OF Topiramate tablets should be NMT2.0%. The %assay of Topiramate should be between 98.0% and 102.0%.

OBSERVATION
The results obtained by comparing with both two types were within limit. Refer tables: 3 &9

5.3.7 ROBUSTNESS
a) Effect of variation of flow rate
A study was conducted to determine the effect of variation in flow rate. Standard solution prepared as per the test method was injected into the HPLC system using flow rates, 1.0ml/min and 1.2ml/min. The system suitability parameters were evaluated and found to be within the limits for 1.0ml/min and 1.2ml/min flow.

Topiramate was resolved from all other peaks and the retention times were comparable with those obtained for mobile phase having flow rates 1.0ml/min.

ACCEPTANCE CRITERIA
The Tailing Factor of Topiramate standards should be NMT 2.0 for Variation in Flow.

OBSERVATION
The tailing factor for MF was found to be within the limits. As shown in table 10.

b) Effect of variation of temperature
A study was conducted to determine the effect of variation in temperature. Standard solution prepared as per the test method was injected into the HPLC system at 20ºC temperature. The system suitability parameters were evaluated and found to be within the limits for a temperature change of 20ºc.

Similarly sample solution was chromatographed at 25ºC temperature. MFH were resolved from all other peaks and the retention times were comparable with those

ACCEPTANCE CRITERIA
The Tailing Factor of Topiramate standard and sample solutions should be NMT 2.0 for Variation in temperature.

OBSERVATION
The tailing factor for Topiramate is found to be within the limits. As shown in table 11.

5.3.8 LIMIT OF DETECTION AND QUANTITATION (LOD and LOQ)
From the linearity data calculate the limit of detection and quantitation, using the following formula.

$$LOD = \frac{3.3\,\sigma}{S}$$

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

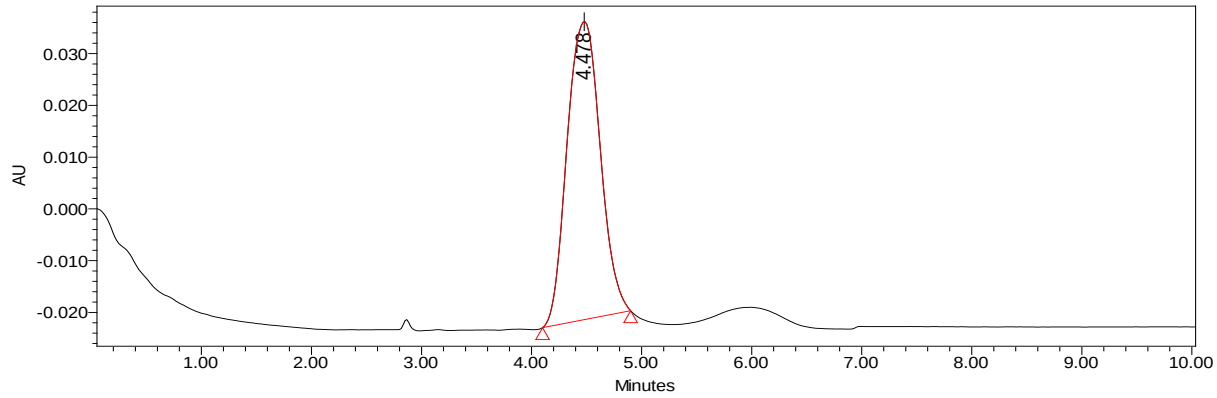
$$LOQ = \frac{10\,\sigma}{S}$$

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

RESULTS

Method development

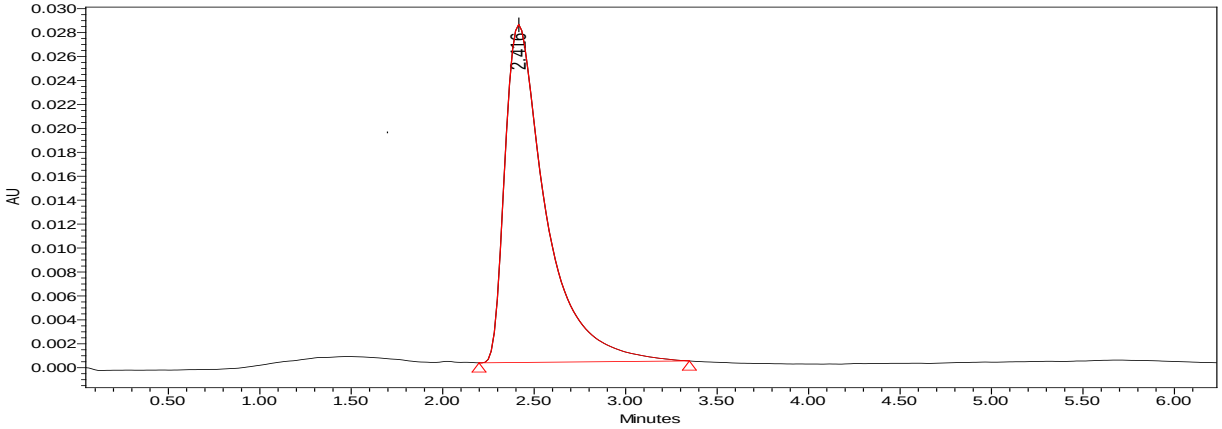
Fig1: Chromatogram of Trial 1



Inference : Theoretical plates are less, peak shape is not good and asymmetry is more than limit.

S.NO	Name of the peak	Retention time(min)
1.	Topiramate	4.428

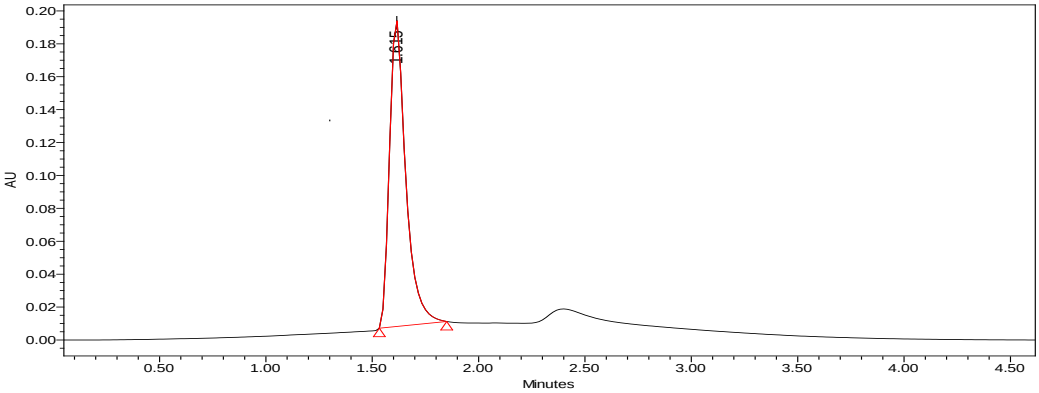
Fig 2: Chromatogram of Trial 2:



Inference : Got more tailing.

S.NO	Name of the peak	Retention time(min)
1	Topiramate	2.418

Fig 3: Chromatogram of Trial3

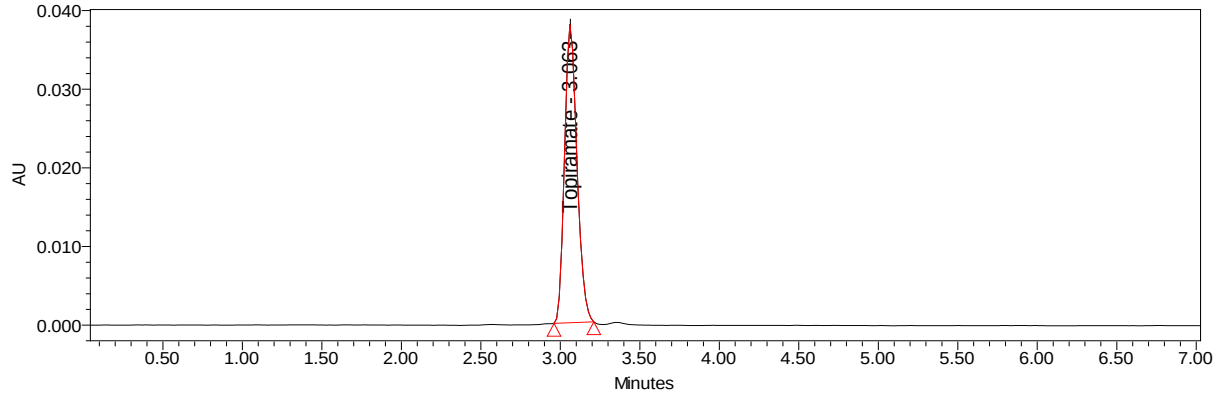


Inference : Got lesss retenton time and base line not good.

S.NO	Name of the peak	Retention time(min)
1.	Topiramate	1.815

6.2 OPTIMIZED METHOD

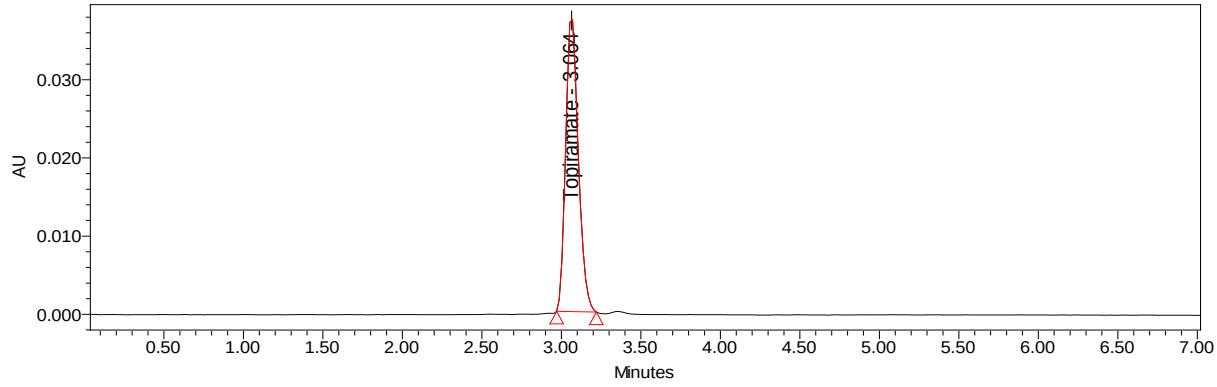
Fig 4: Chromatogram of standard



Inference : Got chromatogram at an Rt of 3.063 for standard

S.NO	Name of the peak	Retention time(min)
1	**Topiramate**	3.063

Fig5:Chromatogram of sample



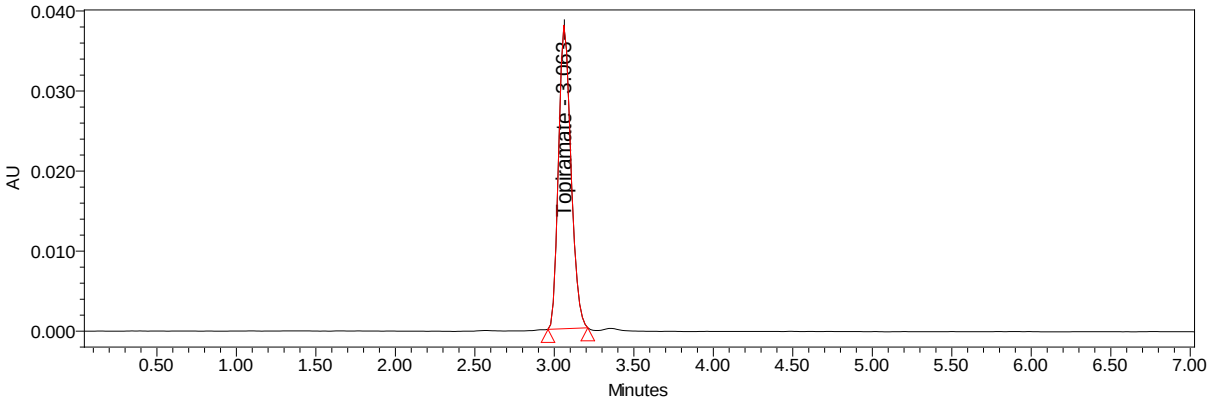
Inference : Got same peak with same Rt 3.064 as of standard.

S.NO	Name of the peak	Retention time(min)
1.	**Topiramate**	3.064

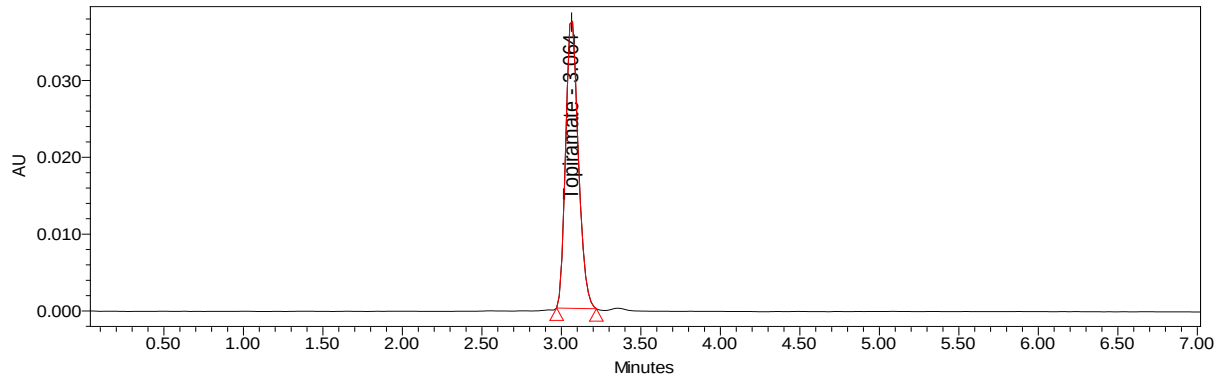
6.3 VALIDATION DATA

6.3.1 SYSTEM SUITABILITY:

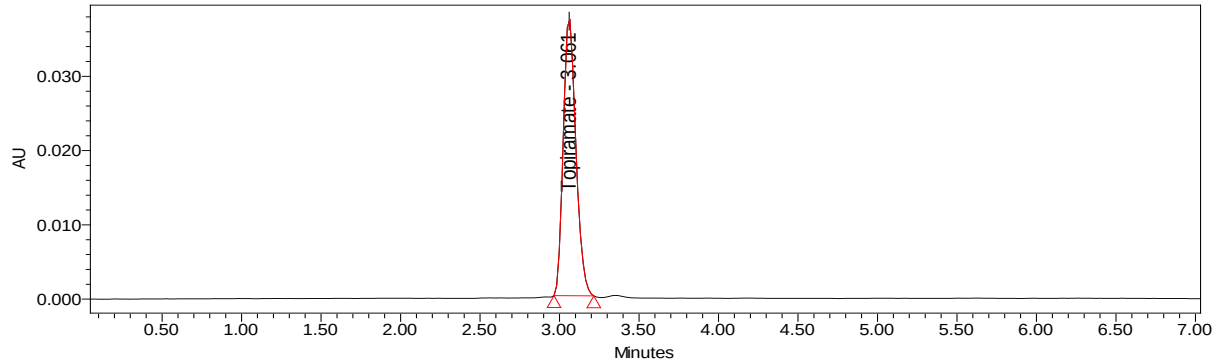
Fig: 6-10 Chromatograms of system suitability(standards 1-5)



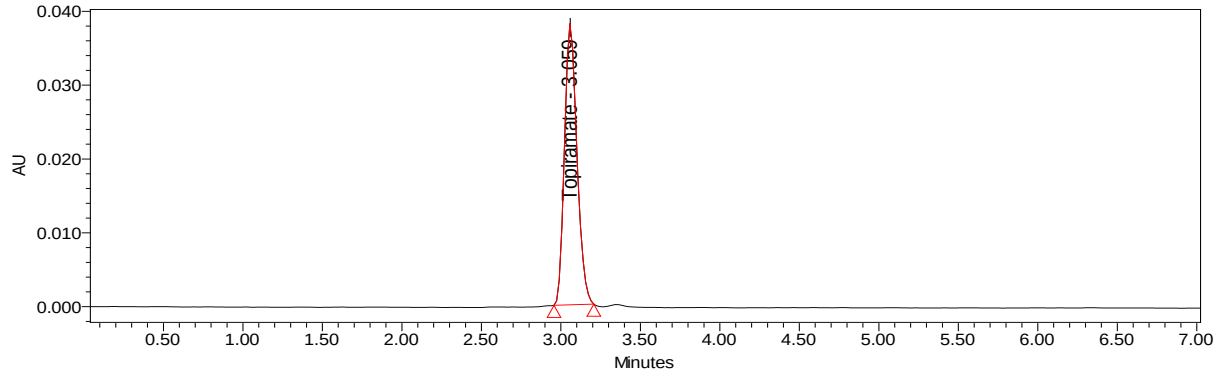
Inference : System sutabilty Chromatogram for standard – 1



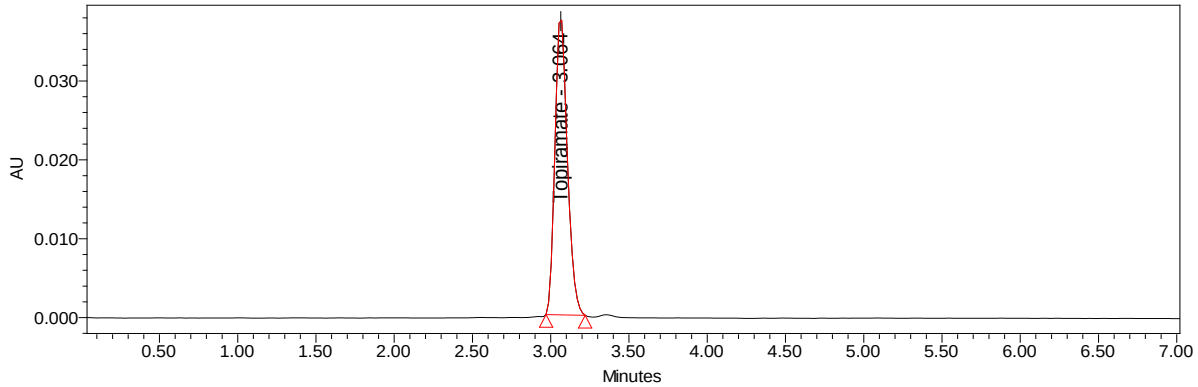
Inference : System sutabilty Chromatogram for standard – 2



Inference : System sutabilty Chromatogram for standard – 3



Inference : System sutabilty Chromatogram for standard - 4



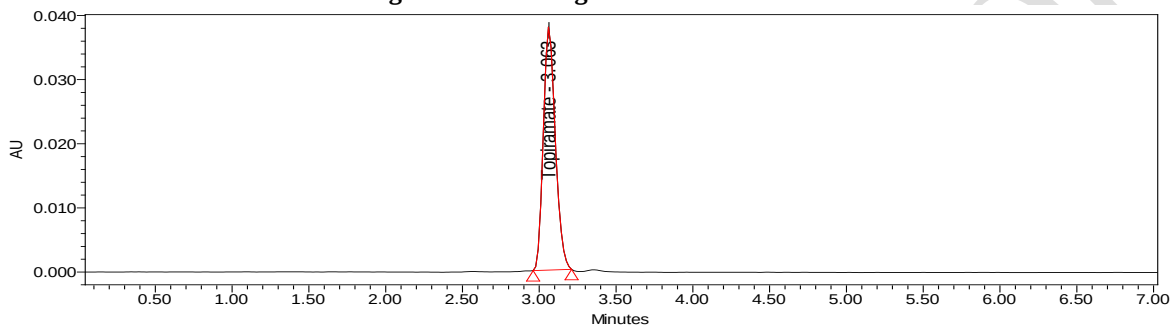
Inference: System sutabilty Chromatogram for standard – 5

TABLE-1: Data of System Suitability

Injection	RT	Peak Area	USP Plate count	USP Tailing
1	3.063	674753	10953.609752	1.153539
2	3.064	674261	10951.014286	1.155271
3	3.061	675298	10003.278630	1.157740
4	3.059	679221	10986.906427	1.159499
5	3.064	688636	10946.878423	1.152820
Mean	3.064201	678433.8	10768.34	1.155774
SD	0.001817	6031.135	-------	-------
% RSD	0.05221	0.888979	-------	-------

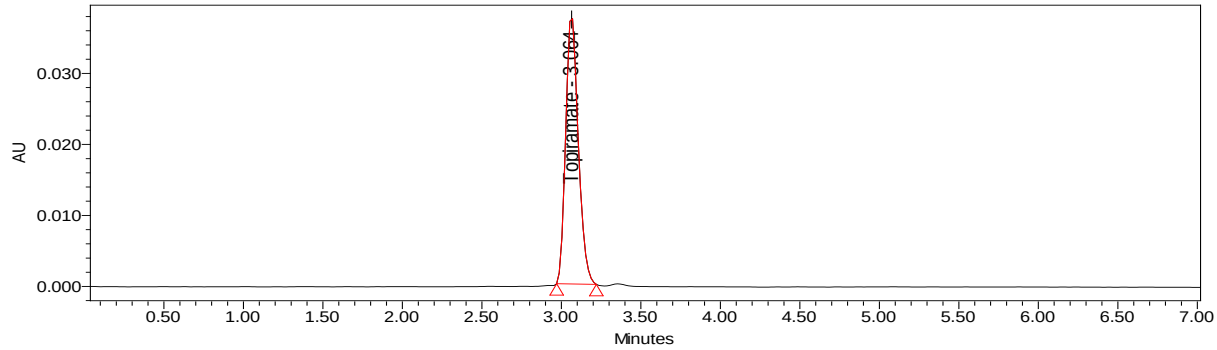
6.3.2: SPECIFICITY

Fig 11: Chromatogram of standard



Inference : Got a peak for standard at an Rt of 3.063

Fig 12: Chromatogram of sample

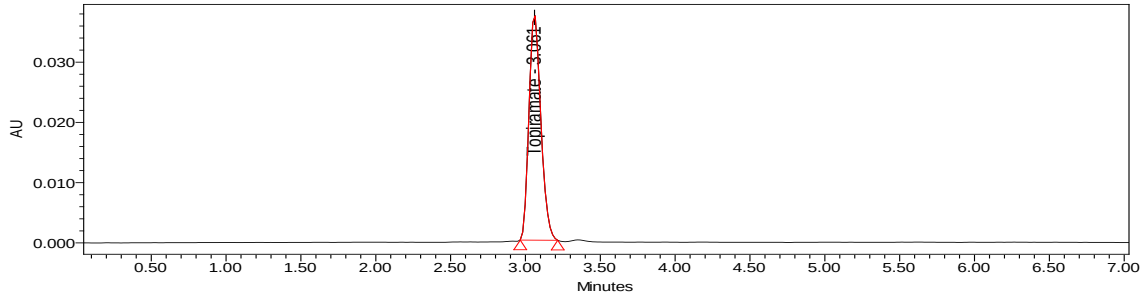


Inference : Got a peak for sample at an Rt of 3.0

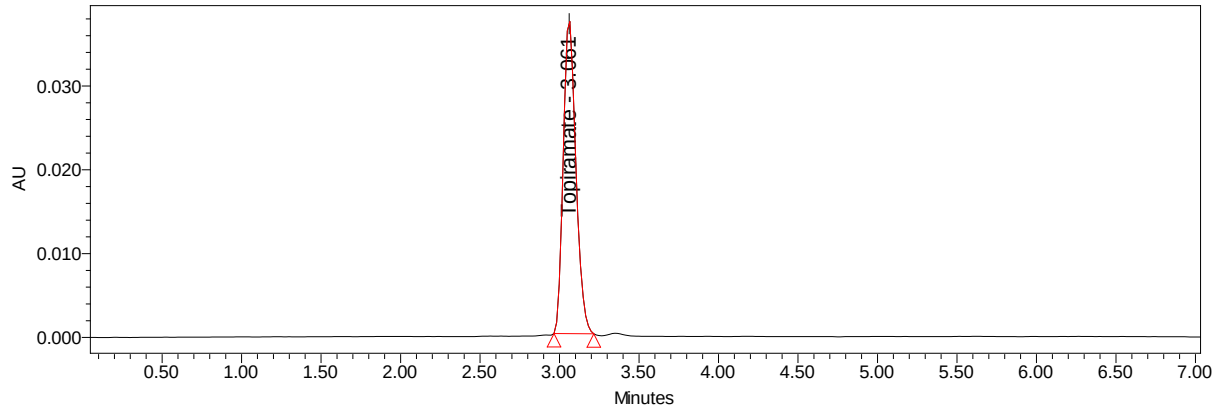
6.3.2: PRECISION
6.3.2.1Repeatability
(a)system precision

Fig13-14 Chromatograms of system precision

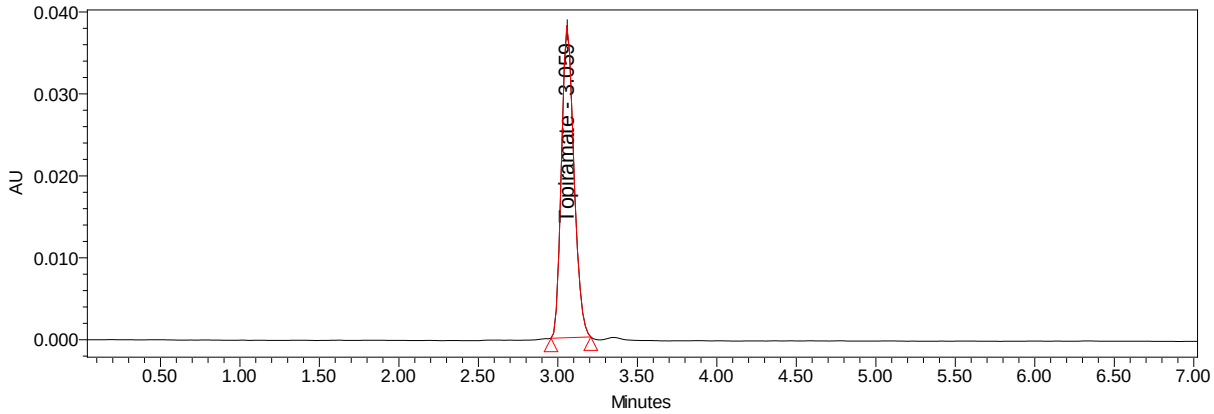
Inference : Chromatogram for system precision (standard - 1)



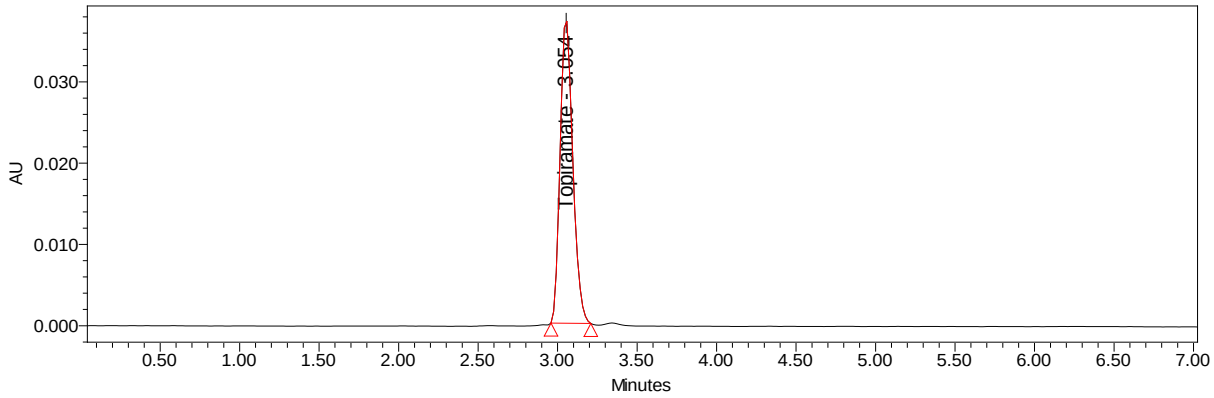
Inference : Chromatogram for system precision (standard - 2)



Inference : Chromatogram for system precision (standard - 3)



Inference : Chromatogram for system precision (standard - 4)



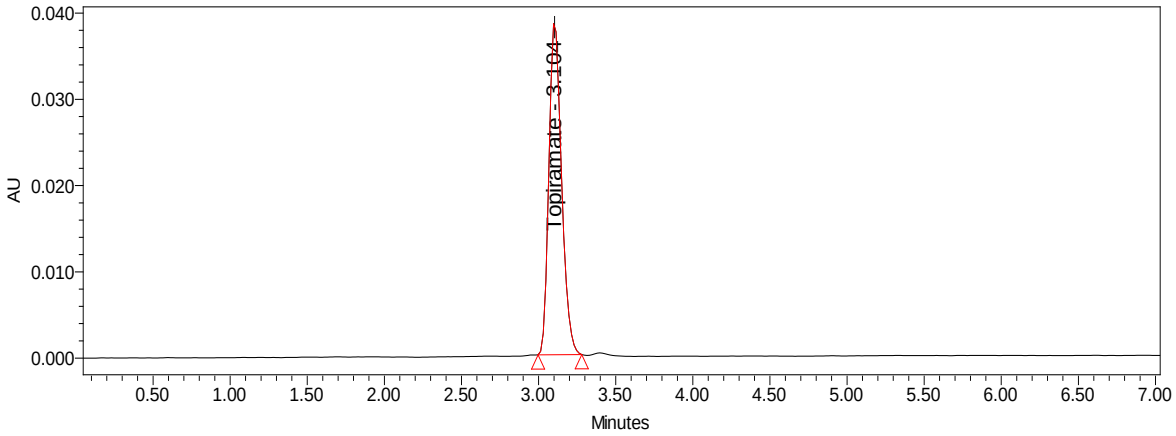
Inference : Chromatogram for system precision (standard - 5)

TABLE-2 Data of Repeatability (System precision)

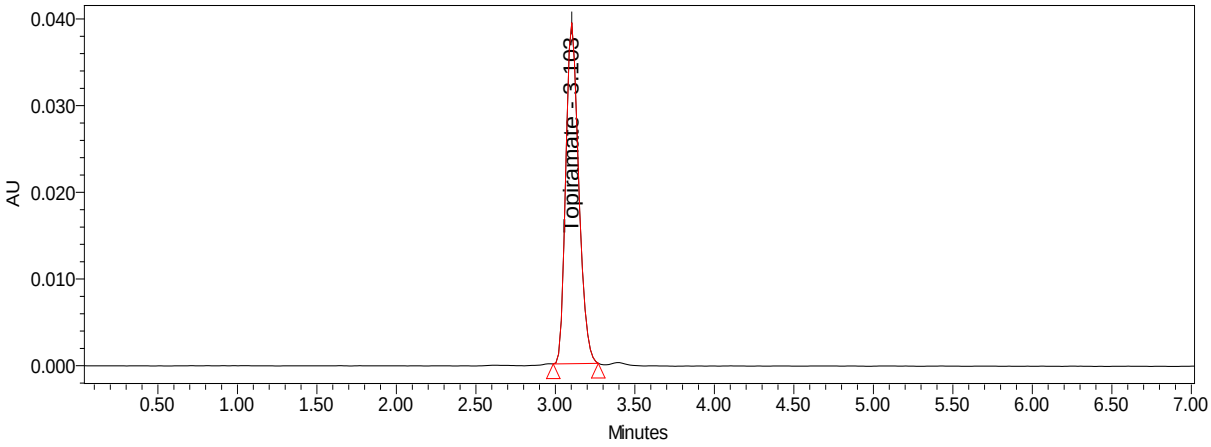
	Injection	Peak Areas of Topiramate	%Assay
Concentration 40ppm	1	674753	98.66
	2	674261	99.30
	3	675298	101.53
	4	679221	100.53
	5	688636	99.98
Statistical Analysis	**Mean**	678433.8	100.00
	SD	6031.135	1.107678
	% RSD	0.888979	1.10

(b)Method precision

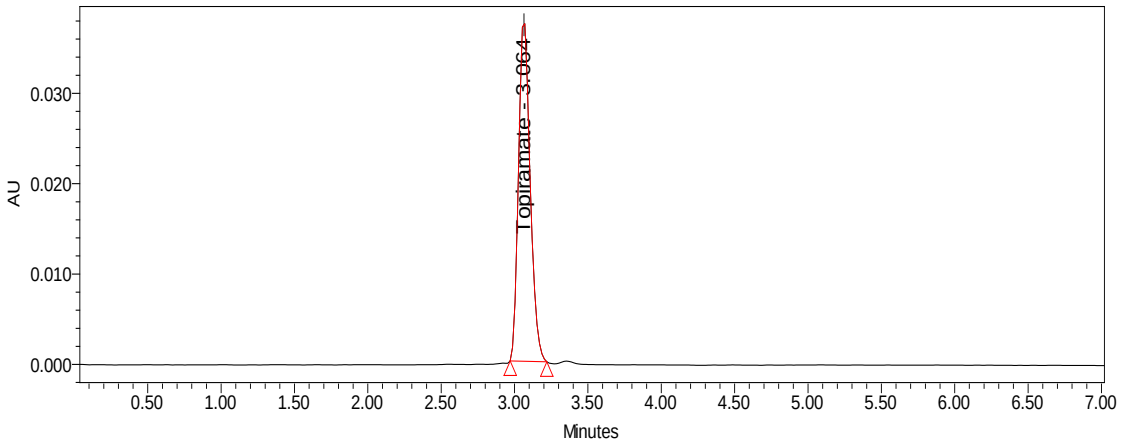
Fig 15-17: Chromatograms of Repeatability



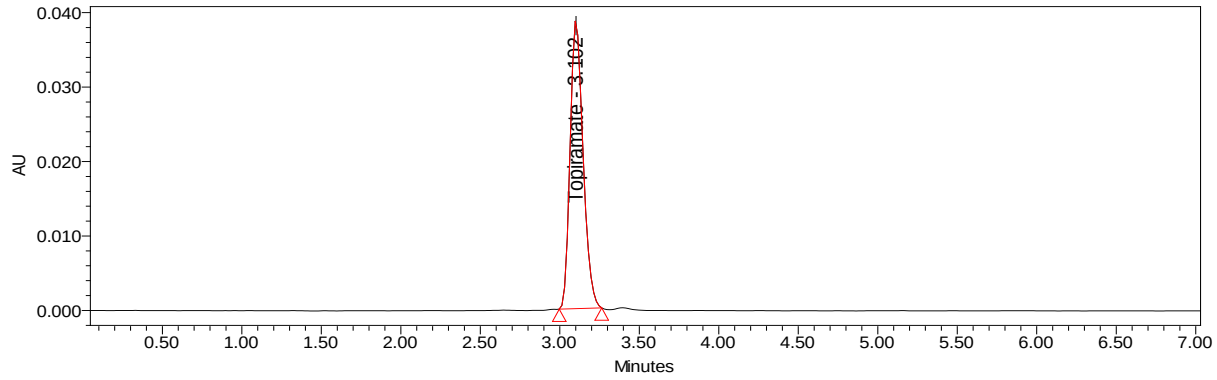
Inference : Chromatogram for Repetability (standard - 1)



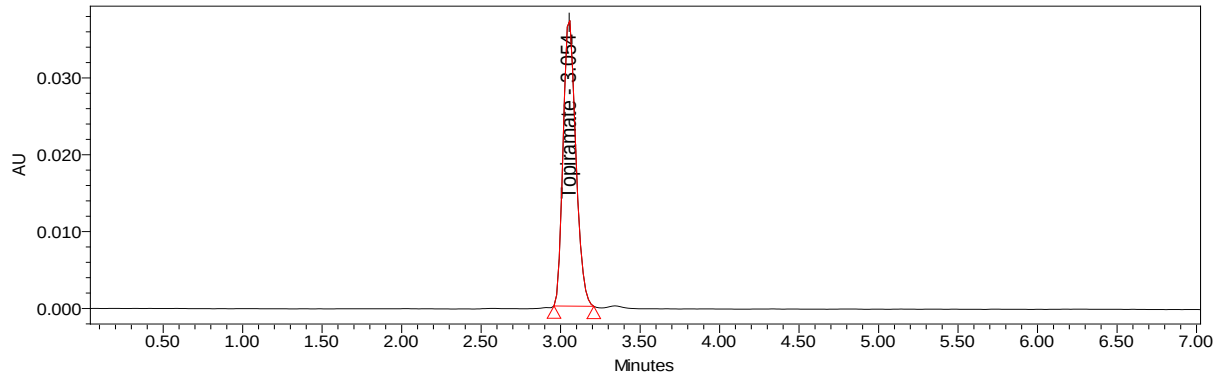
Inference : Chromatogram for Repetability (standard - 2)



Inference : Chromatogram for Repetability (standard - 3)



Inference : Chromatogram for Repetability (standard - 4)



Inference : Chromatogram for Repetability (standard - 5)

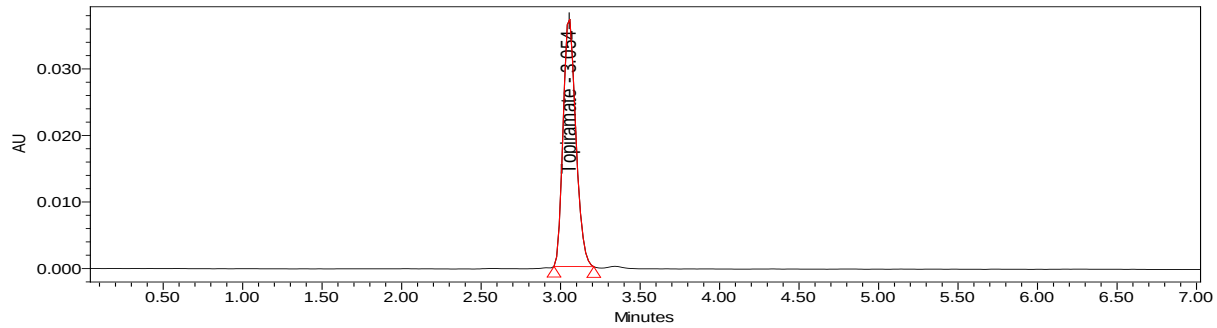
6.3.2.2 Intermediate precision:

For **Analyst 1** ref: Table3. note:(take third table as analyst :**1**

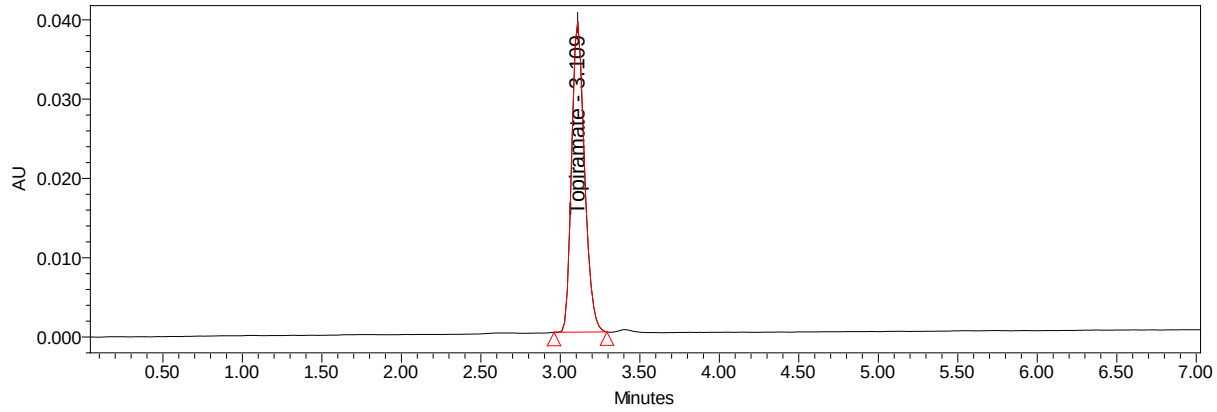
Table4: Data of Intermediate precision (Analyst 2)

	Injection	Peak Areas of Topiramate	%Assay
Concentration 40ppm	1	636792	99.99
	2	634360	99.66
	3	655696	101.53
	4	644147	99.98
	5	644127	99.97
	6	652525	101.10
Statistical Analysis	**Mean**	644607.8	100.37
	SD	6392.59	0.753536
	% RSD	1.183	0.75

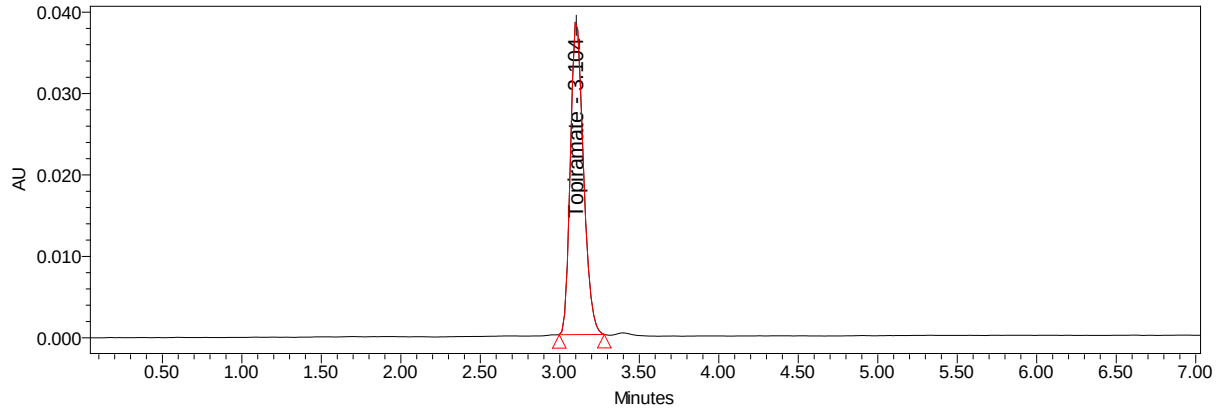
Fig18-20: Chromatograms of Intermediate precision



Inference : Chromatogram for Intermedate Precsion



Inference : Chromatogram for Intermedate Precsion



Inference : Chromatogram for Intermedate Precsion

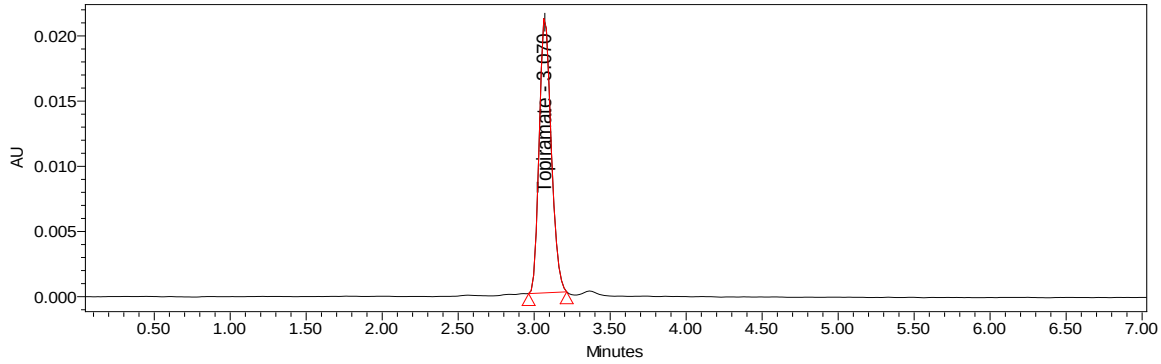
6.3.4 ACCURACY (RECOVERY)

TABLE-5: Data of Accuracy

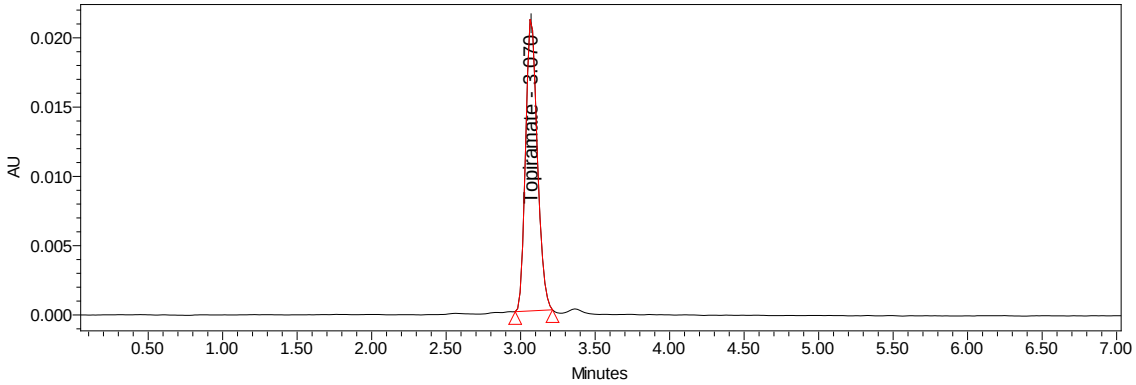
Concentration % of spiked level	Amount added (ppm)	Amount found (ppm)	% Recovery	Statistical Analysis of % Recovery	
50% Injection 1	20	20.04	100.22	**MEAN**	100.06
50% Injection 2	20	19.97	99.85		
50% Injection 3	20	20.02	100.11	**%RSD**	0.18
100 % Injection 1	40	40.01	100.02	**MEAN**	100.04
100 % Injection 2	40	40.05	100.14		
100% Injection 3	40	39.98	99.96	**%RSD**	0.091
150% Injection 1	60	60.08	100.14	**MEAN**	100.02
150% Injection 2	60	59.97	99.96		
150% Injection 3	60	59.98	99.98	**%RSD**	0.09

EJPMR

Fig21 -22Chromatograms for accuracy (50%)

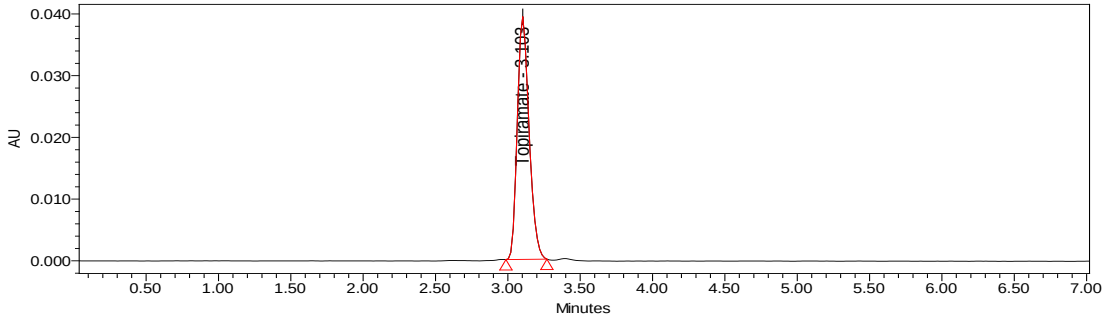


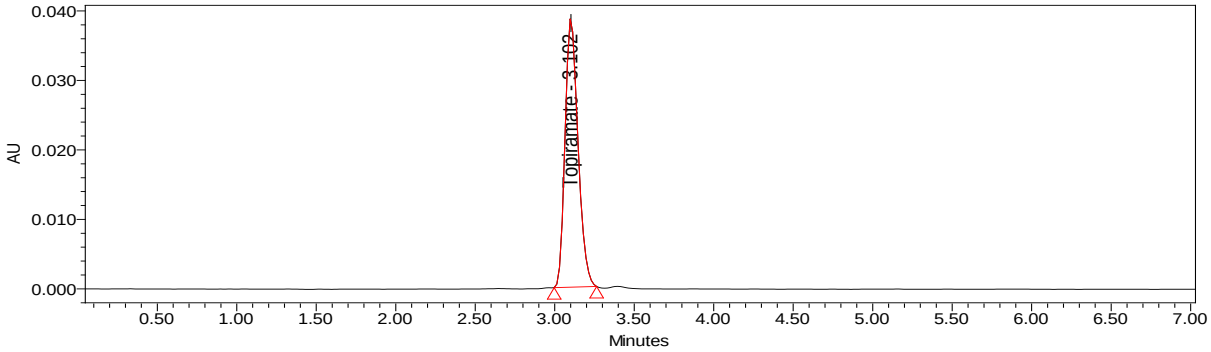
Inference : Chromatogram for standard 1



Inference : Chromatogram for standard 2

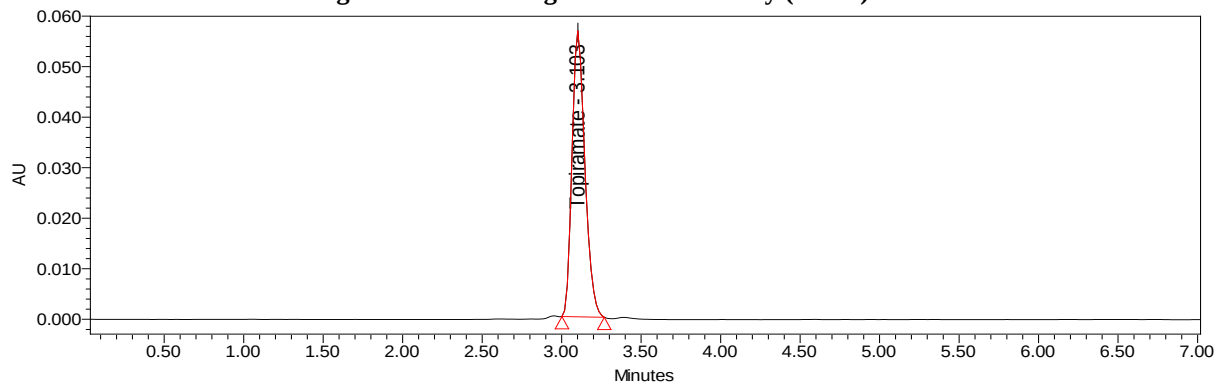
Fig 23-24: Chromatograms for accuracy (100%)



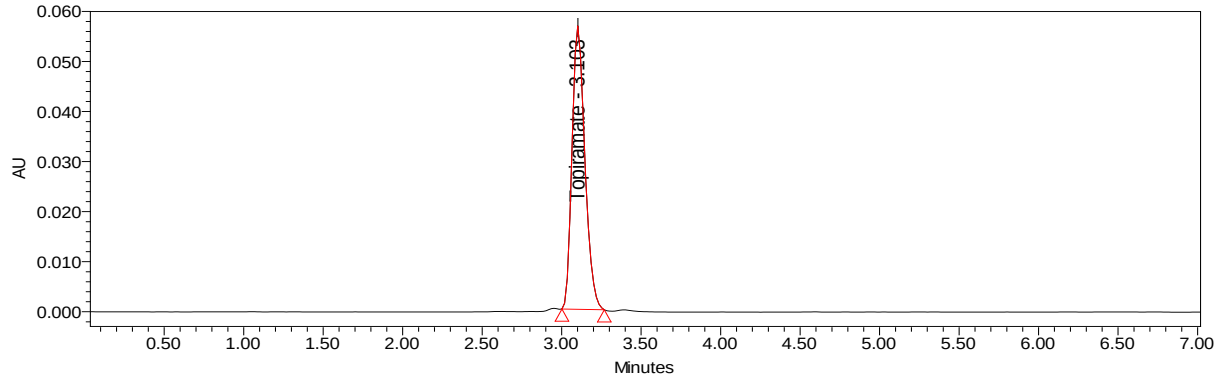


Inference : Chromatogram for standard 2

Fig 25-26: chromatograms For Accuracy (150%)



Inference : Chromatogram for standard 1



Inference : Chromatogram for standard 2

6.3.5 LINEARITY

TABLE6: Data of Linearity

Concentration (ppm)	Average Area	Statistical Analysis	
0	0	Slope	18600
20	632546	y-Intercept	276.2
30	658296	Correlation Coefficient	1
40	694400		
50	730308		
60	916282		
70	9402046		
80	9788277		

Fig: 41 Linearity Plot (Concentration Vs Response)

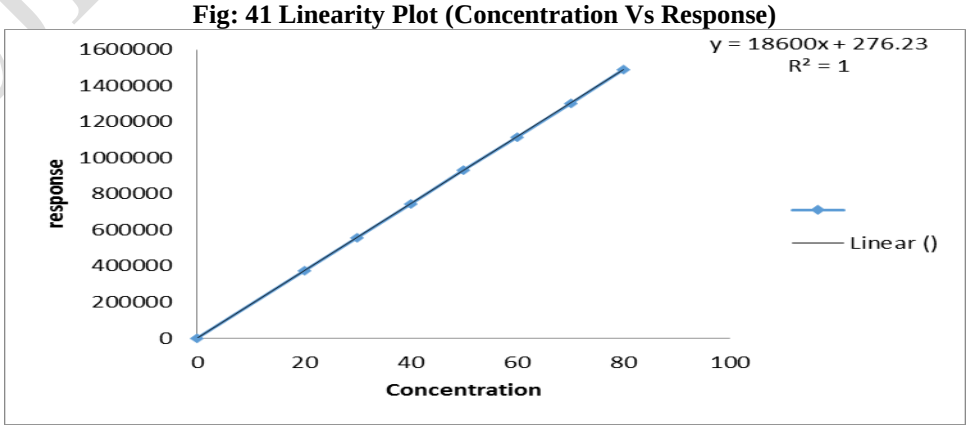
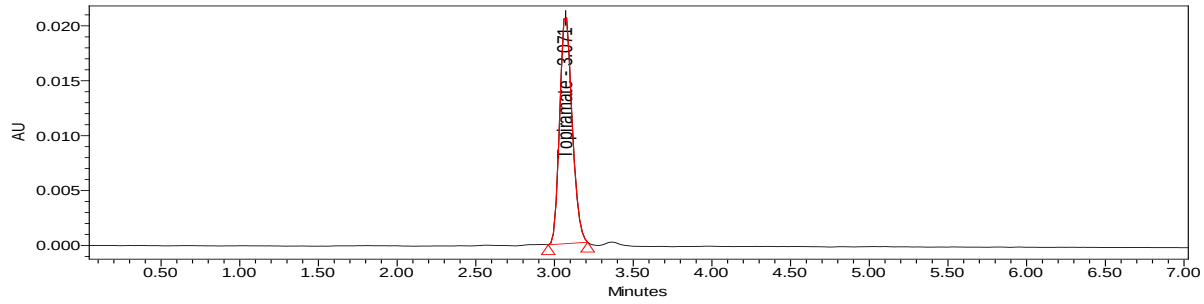
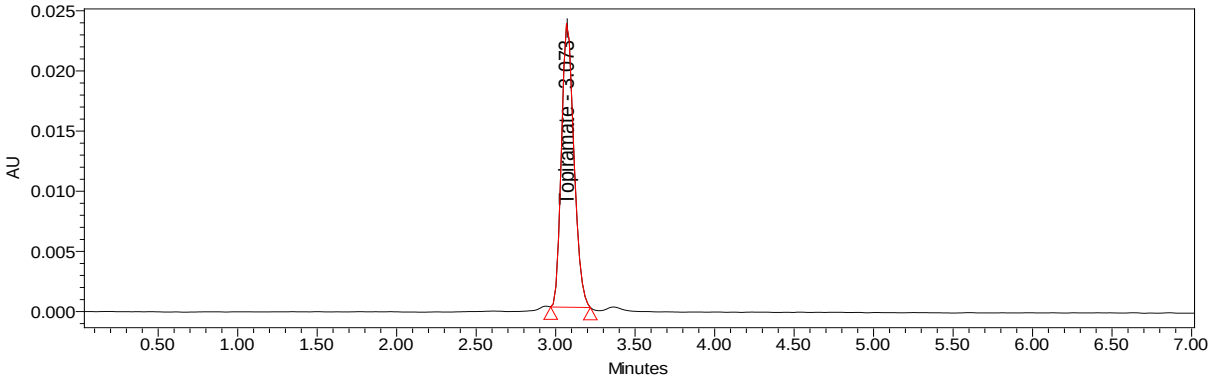


Fig:27-28Chromatograms for 20 ppm

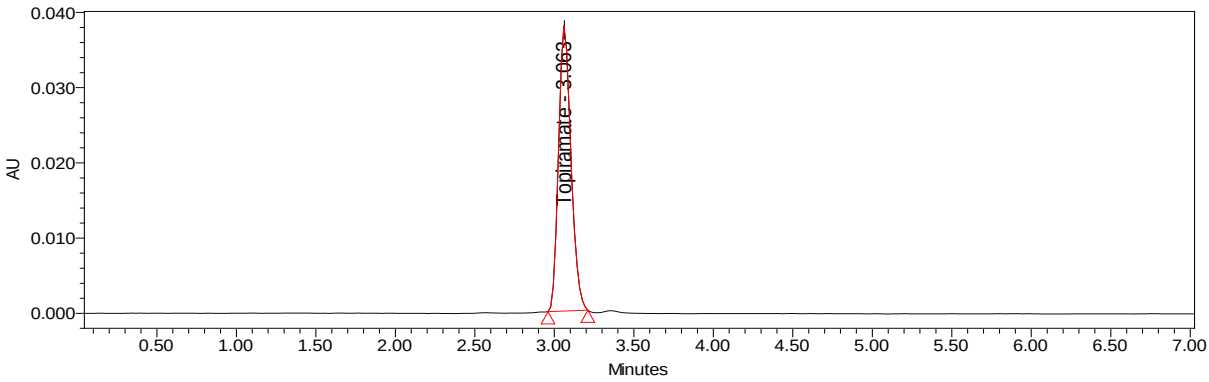


Inference: Chromatogram for 20 ppm standard 1

Fig: 29-30 chromatograms for 30ppm, 40 ppm

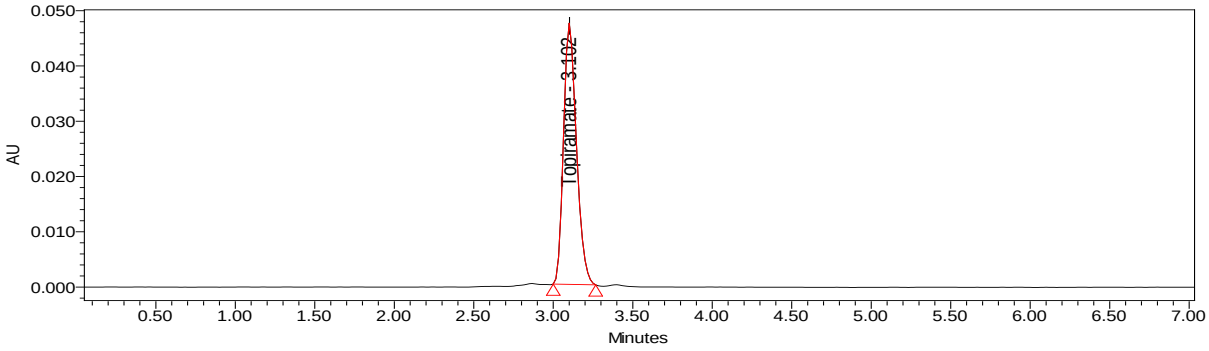


Inference: Chromatogram for 30 ppm standard 1

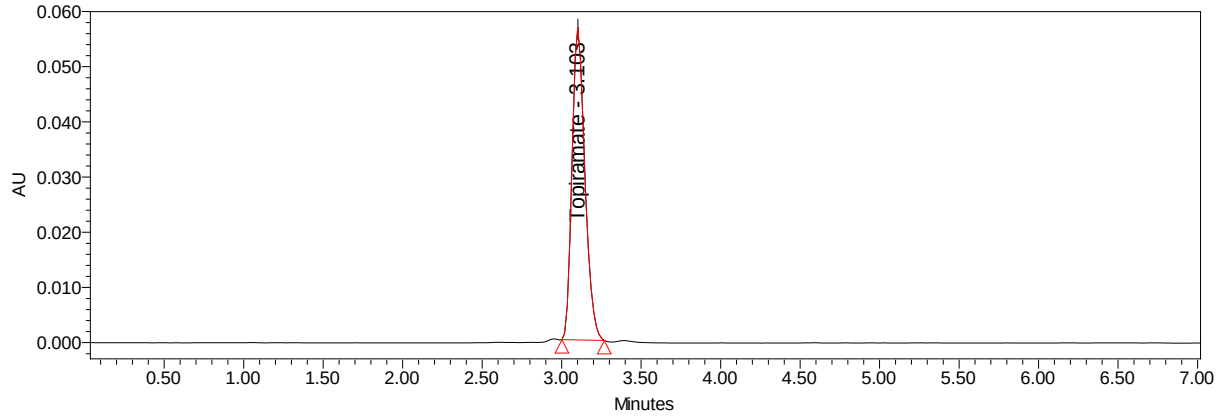


Inference: Chromatogram for 40 ppm standard 1

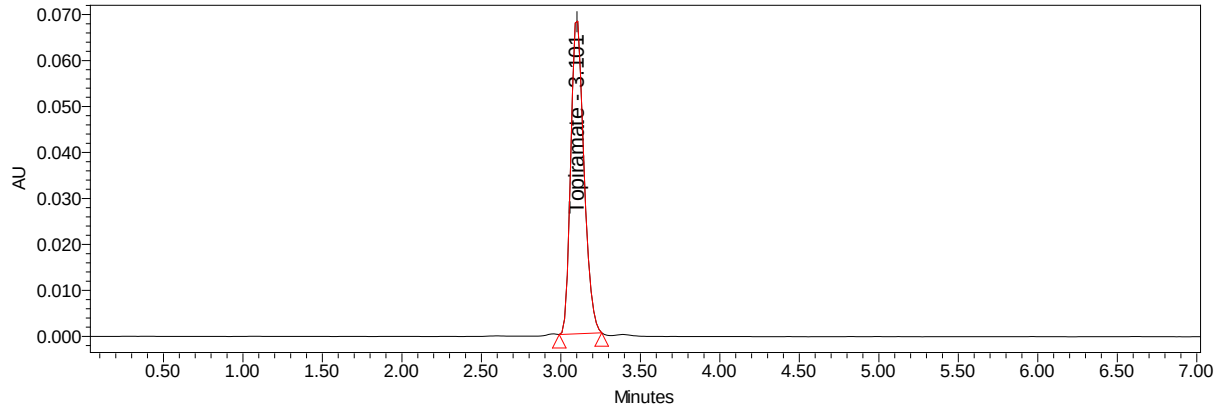
Fig31:32 Chromatograms for 50 ppm, 60 ppm



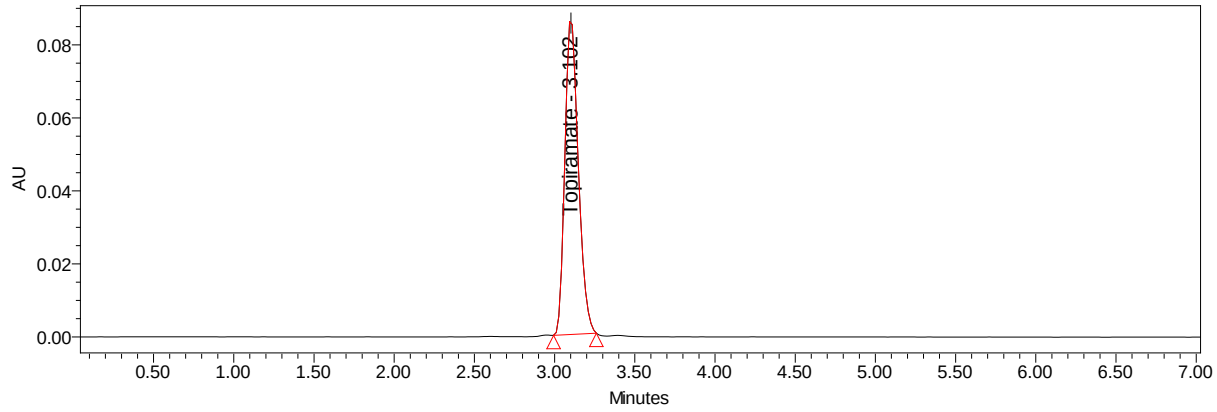
Inference: Chromatogram for 50 ppm standard 1



Inference: Chromatogram for 60 ppm standard 1



Inference: Chromatogram for 70 ppm standard 1



Inference: Chromatogram for 80 ppm standard 1

6.3.6 Ruggedness

a)System to System variability

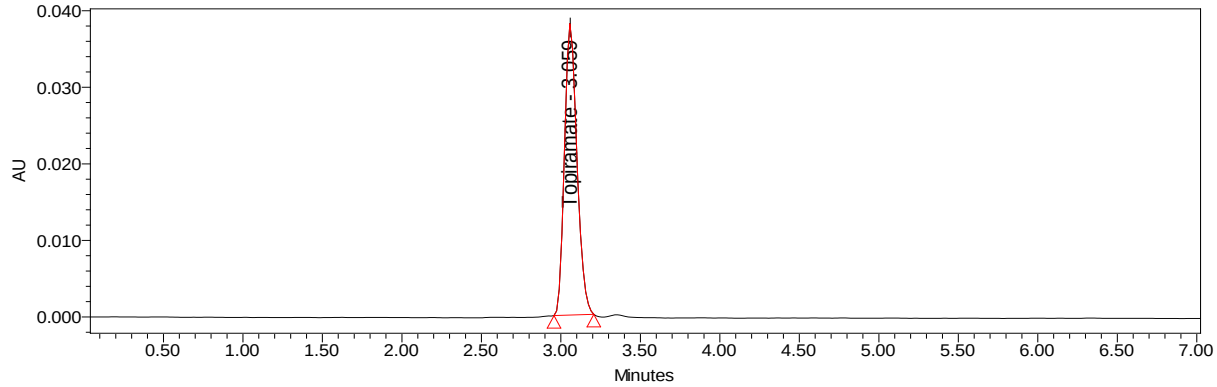
For **system 1** Refer: Table3

TABLE: 7 Data of system to system variability (sample)

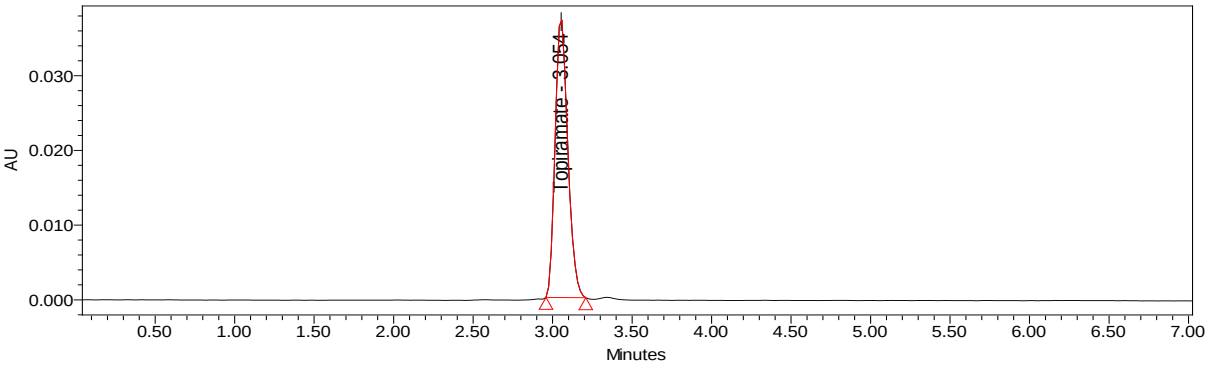
System-2

S.NO:	Peak area	Assay % of Topiramate
1	634360	98.65
2	634098	98.63
3	635696	98.86
4	633289	98.52
5	634147	98.63
6	633495	98.55
Mean	634180.8	98.64
%RSD	0.019	0.12

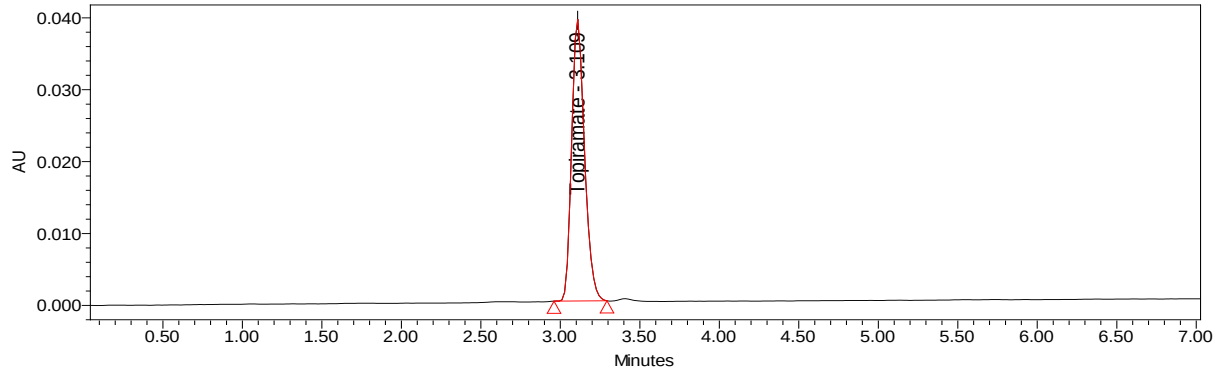
Chromatograms of system to system variability



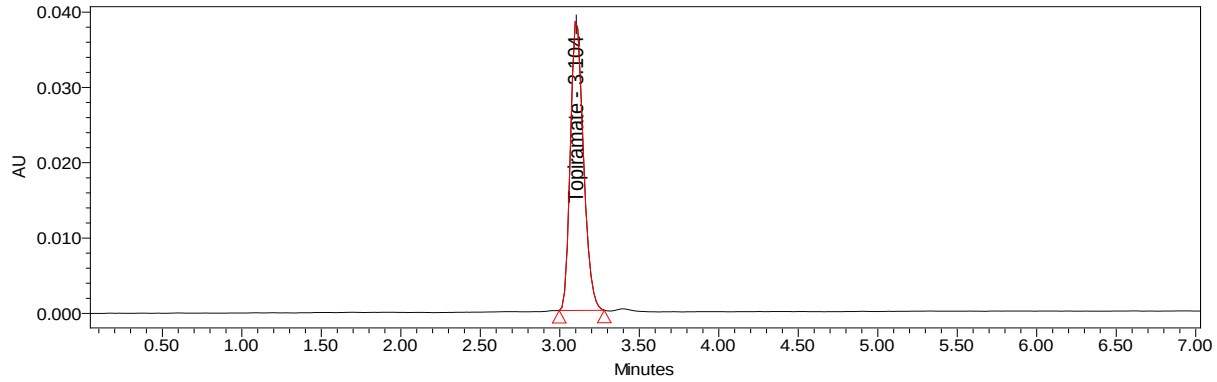
Inference: Chromatogram of system to system variability std- 1



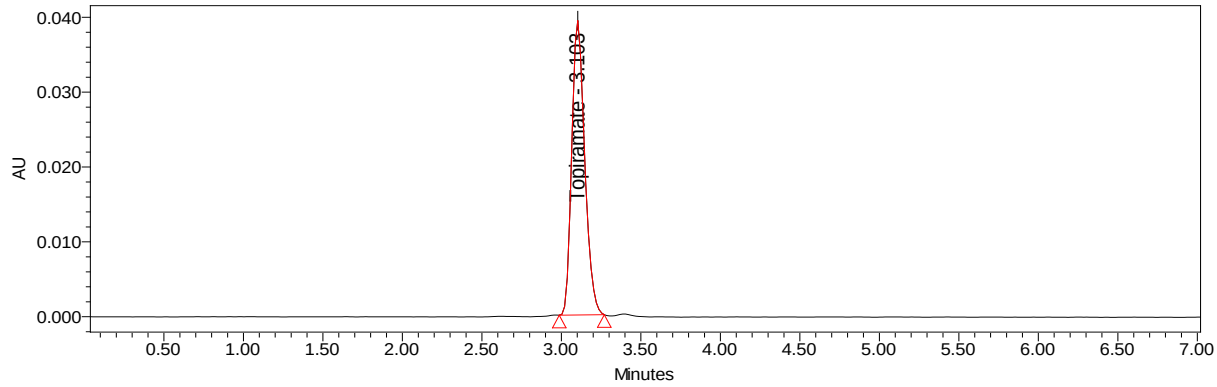
Inference: Chromatogram of system to system variability std- 2



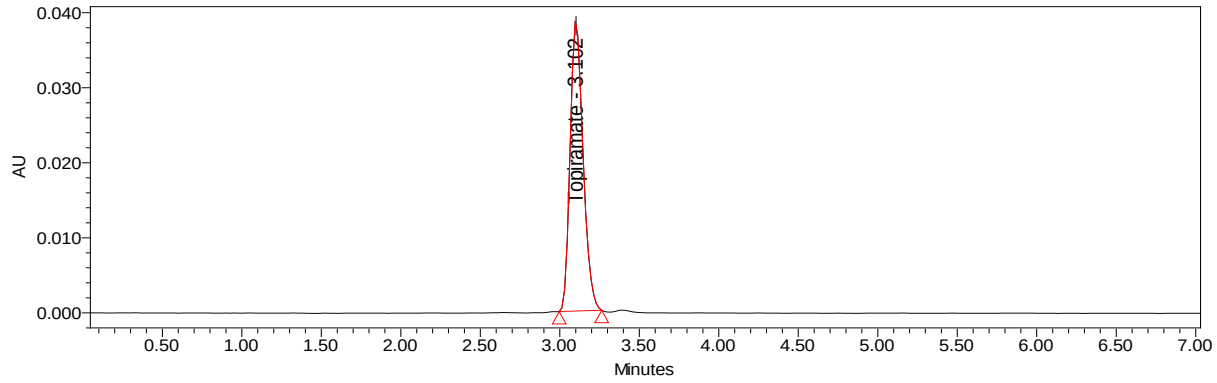
Inference: Chromatogram of system to system variability std- 3



Inference: Chromatogram of system to system variability std- 4



Inference: Chromatogram of system to system variability std- 5



Inference: Chromatogram of system to system variability std- 6

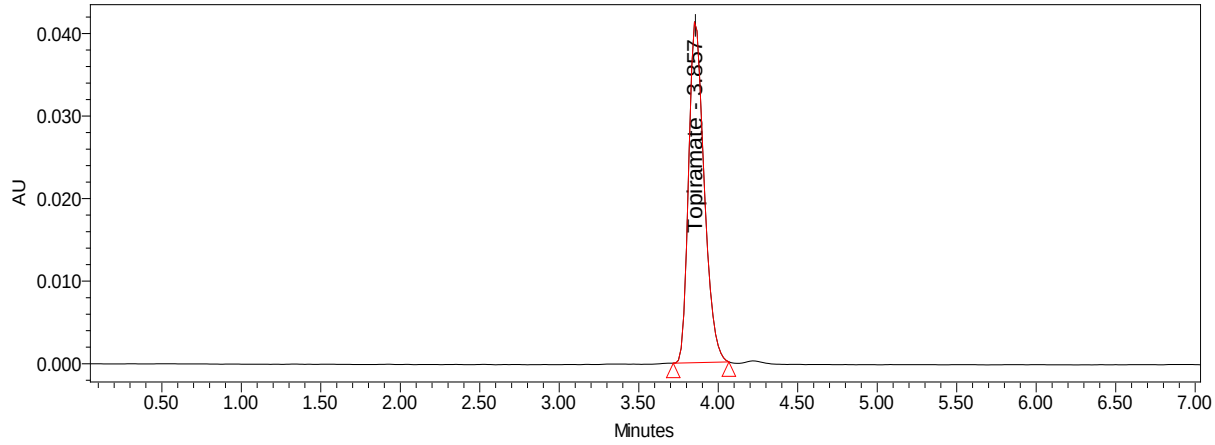
6.3.7 Robustness

TABLE: 10 Data for Effect of variation in flow rate

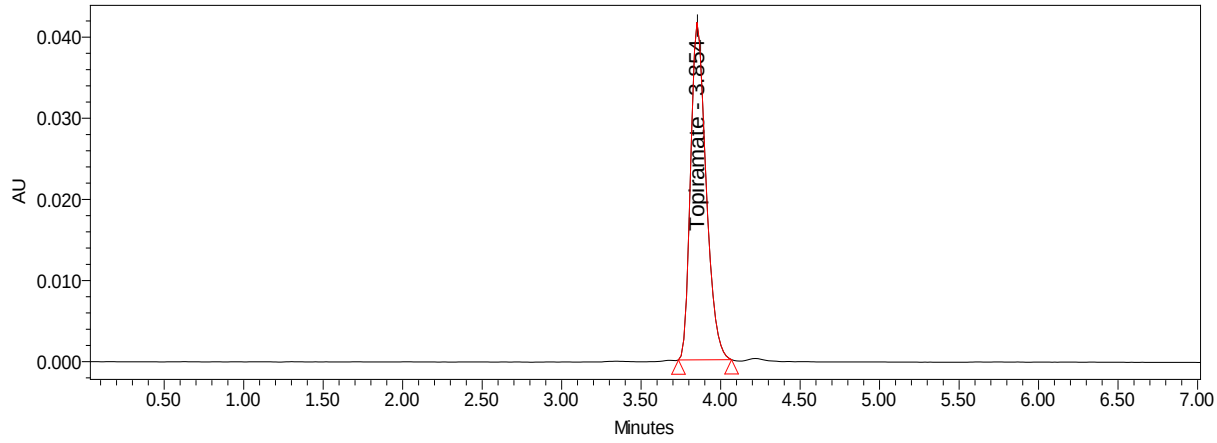
Flow 0.8 ml	Std Area	Tailing factor	Flow 1.0 ml	Std Area	Tailing factor	Flow 1.2 ml	Std Area	Tailing factor
	620286	1.322089		634322	1.604878		602077	1.285372
	619282	1.331920		635792	1.584354		601854	1.319385
	621337	1.296438		634360	1.543805		602403	1.292055
	620456	1.315454		635696	1.568590		603421	1.304561
	620765	1.326551		633147	1.559986		602465	1.294621
Avg	620425	1.31849	**Avg**	634663.4	1.572323	**Avg**	602444	1.299199
SD	754.0018	0.013728	**SD**	1100.917	0.023367	**SD**	599.8833	0.013223
%RSD	0.086	1.04	**%RSD**	0.184	1.48	**%RSD**	0.09	1.01

Fig42-44Chromatograms of robustness

a) Effect of variation of flow rate (for 0.8 ml/min flow)

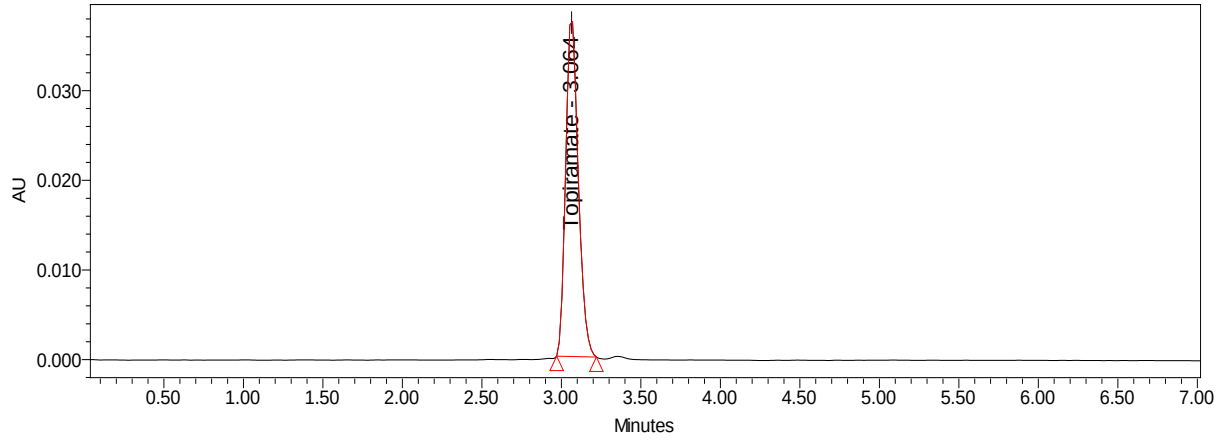


Inference : Chromatogram for robustness standard – 1

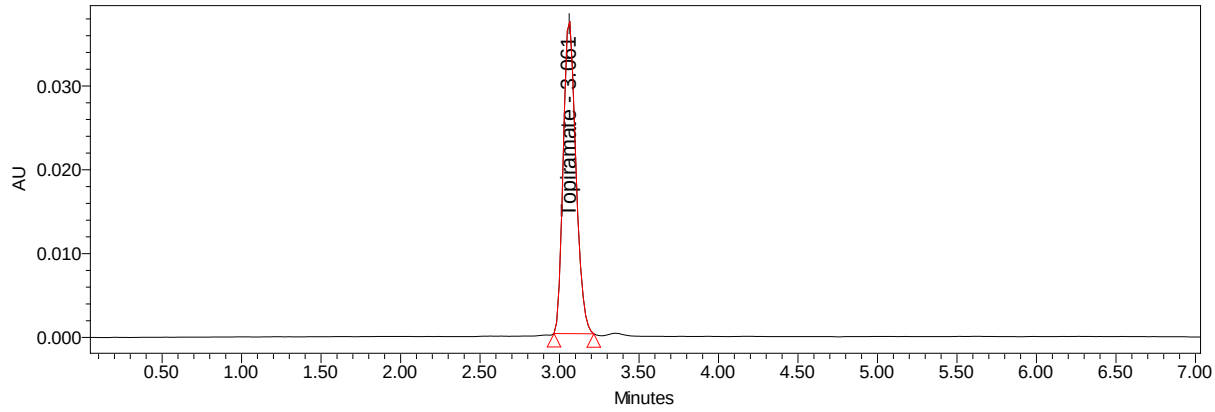


Inference : Chromatogram for robustness standard - 2

Fig45-46: chromatograms for 1ml/min

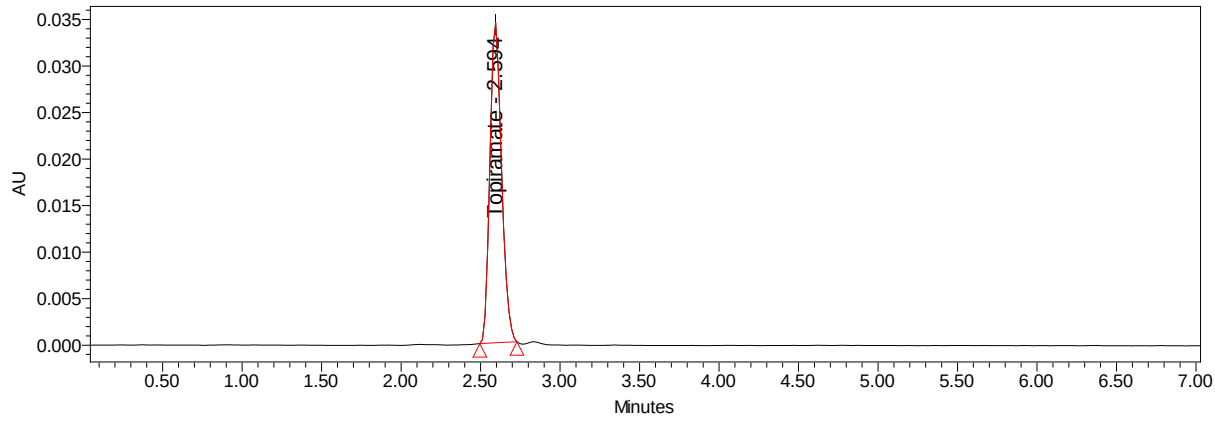


Inference : Chromatogram for robustness standard - 1

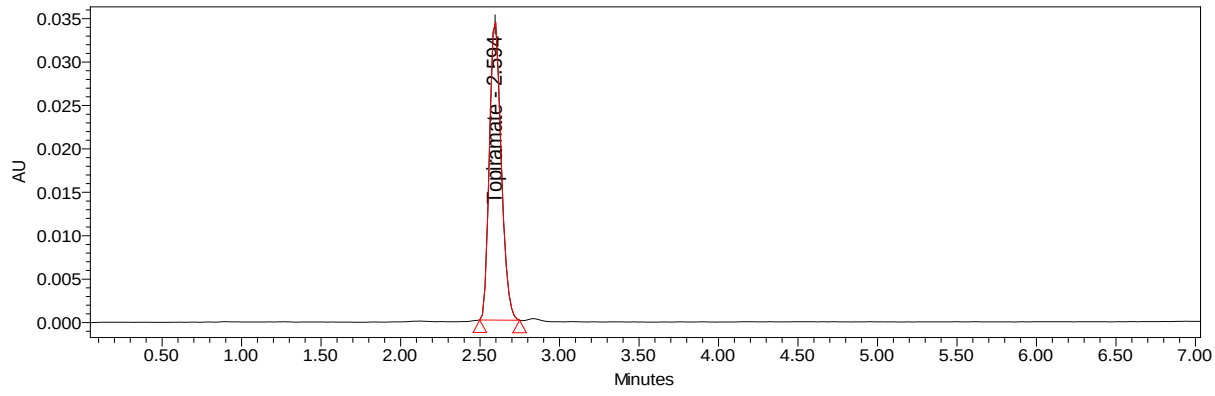


Inference : Chromatogram for robustness standard - 2

Fig47-49: Chromatograms for 1.2ml/min



Inference : Chromatogram for robustness standard – 1



Inference : Chromatogram for robustness standard - 2

6.3.8LIMIT OF DETECTION AND LIMIT OF QUANTITATION (LOD and LOQ):

From the linearity plot the LOD and LOQ are calculated:

$$LOD = \frac{3.3\ \sigma}{S}$$

$$= \frac{3.3 \times 3244.904}{18600} = 0.57$$

$$LOQ = \frac{10\ \sigma}{S}$$

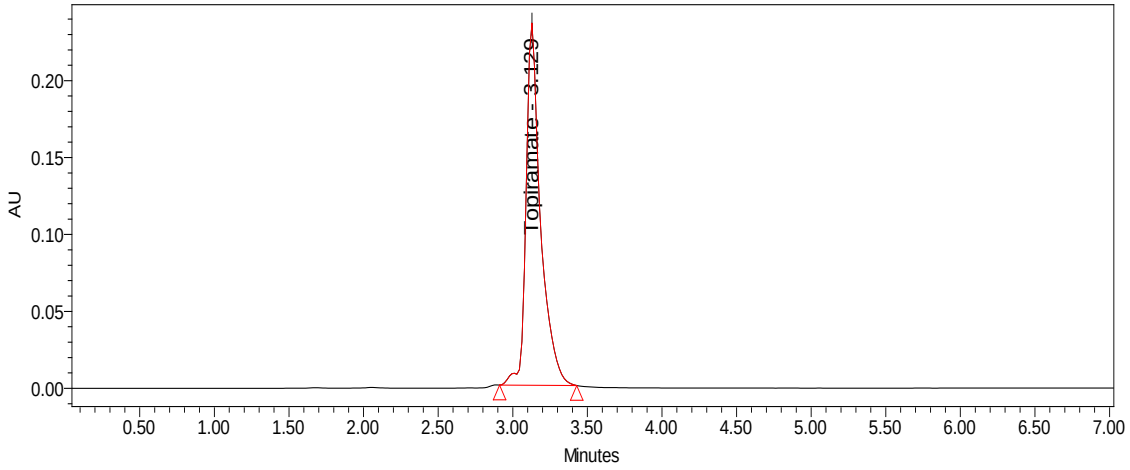
$$= \frac{10 \times 3244.904}{18600} = 1.74$$

STABLITY TESTING RESULTS

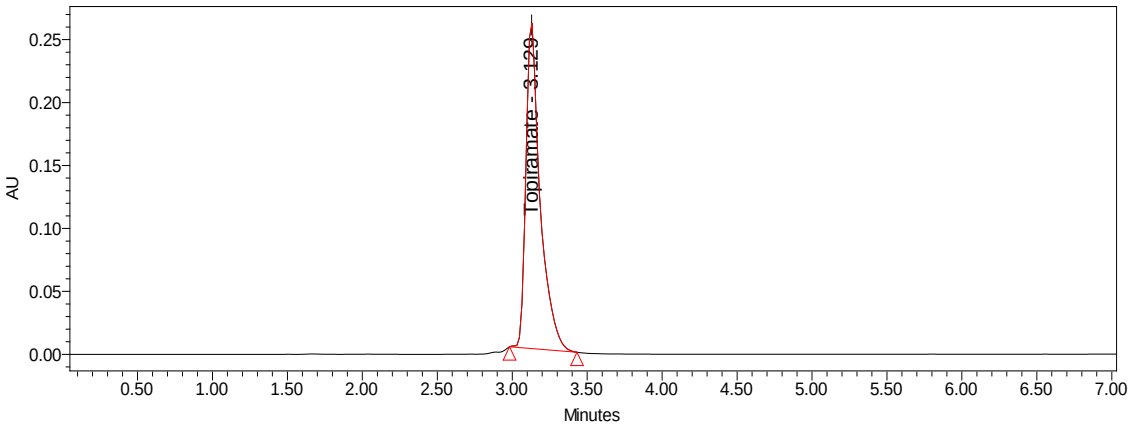
Forced Degradation for Topiramate

Mode of Degradation	Condition	Period	% Assay	% Degradation as compared with Control
Control sample	No treatment	-	98.49	-
Acid	1.0N HCl	10 Minutes	100.25	-1.22
Base	0.05N NaOH	5 Minutes	98.07	0.96
Oxidative	3.0% v/v H_2O_2	1 Hour	85.36	13.67
Thermal	80^0C	12 Hour	100.45	-1.42

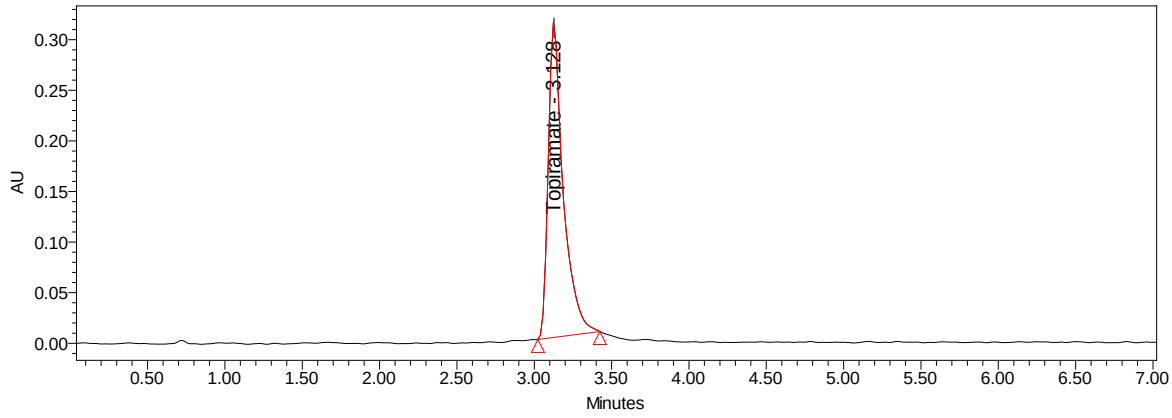
Acid Graph



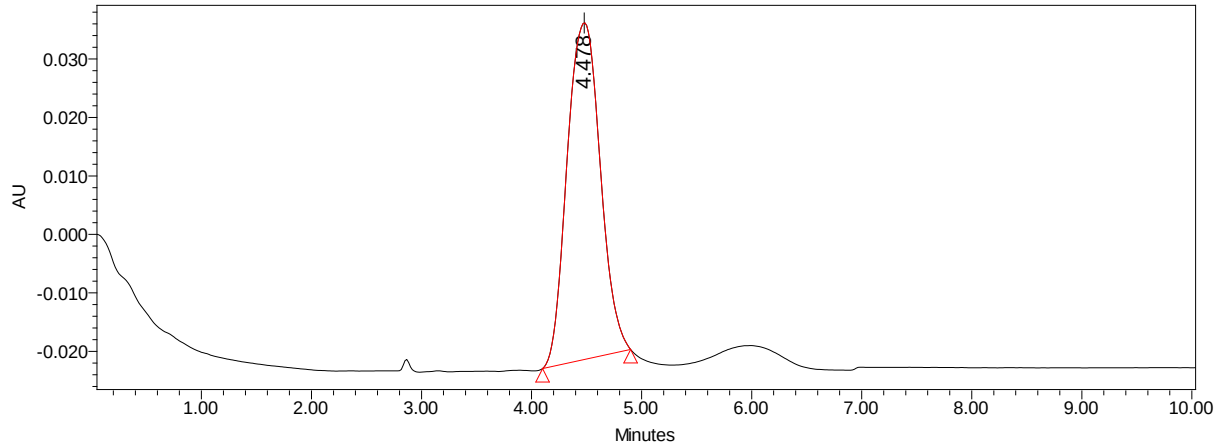
Base Graph



Oxidative Graph



Thermal Graph



CONCLUSION

The analytical method was developed by studying different parameters. First of all, maximum absorbance was found to be at 238nm for Topiramate. The peaks purity was excellent. Injection volume was selected to be 20µl which gave a good peak area. The column used for study was Inertsil C_{18}, ODS chosen good peak shape. Ambient temperature was found to be suitable for the nature of drug solution. The flow rate was fixed at 1.0ml/min because of good peak area, satisfactory retention time and good resolution. Different ratios of mobile phase were studied, mobile phase with ratio of 50:50Methanol: Water was fixed due to good symmetrical peaks and for good resolution. So this mobile phase was used for the proposed study.

The present recovery was found to be 98.0-101.50 was linear and precise over the same range. Both system and method precision was found to be accurate and well within range. Detection limit was found to be 0.56 for Topiramate. The analytical method was found linearity over the range of 20-80ppm of the target concentration for the drugs. The analytical passed both robustness and ruggedness tests. On both cases, relative standard deviation was well satisfactory

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