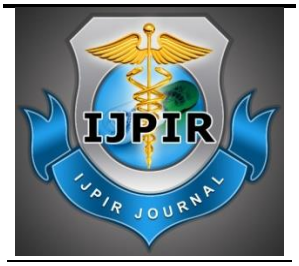
Research Article



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Reverse phase high performance liquid chromatography method for estimation of mexiletine in pure and in injection

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ABSTRACT

RPC operates on the principle of hydrophobic interactions, which result from repulsive forces between a polar eluent, the relatively non-polar analyte, and the non-polar stationary phase.In this work we aimed at develop new simple, sensitive, accurate and economical analytical method for the estimation of Mexiletine & To validate the proposed method in accordance with USP and ICH guidelines for the intended analytical application i.e., to apply the proposed method for analysis of the Mexiletine in dosage form &finally a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Mexiletine in bulk drug and pharmaceutical dosage forms. Mexiletine was freely soluble in ethanol, methanol and sparingly soluble in water. The %RSD values were within 2 and the method was found to be precise. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods.This method can be used for the routine determination of Mexiletine in bulk drug and in Pharmaceutical dosage forms.

Keywords: RP-HPLC, RSD, Mexiletine, Hydroxy methyl mexilitine, PHM.

INTRODUCTION

Analytical chemistry [1] is a scientific discipline used to study the chemical composition, structure and behaviour of matter. An analytical method consists of a detailed, stepwise list of instructions to be followed in the qualitative, quantitative or structural analysis of a sample for one or more analytes and using a specified technique. The chromatography was discovered by Russian Chemist and botanist Micheal Tswett (1872-1919) who first used the term chromatography (colour writing derived from Greek for colour – Chroma , and write – graphein) to describe his work on the separation of coloured plant pigments into bands on a column of chalk and other material such as polysaccharides, sucrose and insulin.“ Chromatography is a method in which the components of a mixture are separated on an adsorbent column in a flowing system".

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Reversed phase HPLC (RP-HPLC) consists of a non-polar stationary phase and an aqueous, moderately polar mobile phase. One common stationary phase is silica which has been treated with RMe2SiCl, where R is a straight chain alkyl group such as C18H37 or C8H17. The retention time is therefore longer for molecules which are more non-polar in nature, allowing polar molecules to elute more readily. Retention Time (Rt) is increased by the addition of polar solvent to the mobile phase and decreased by the addition of more hydrophobic solvent. The pharmaceutical industry regularly employs RPC to qualify drugs before their release.

RPC operates on the principle [5, 6] of hydrophobic interactions, which result from repulsive forces between a polar eluent, the relatively non-polar analyte, and the non-polar stationary phase. The binding of the analyte to the stationary phase is proportional to the contact surface area around the non-polar segment of the analyte molecule upon association with the ligand in the aqueous eluent.

Mexiletine,a drug used to treat chronic pain,abnormal heart rhythms, causes of muscle stiffness, is an analogue of Lidocaine [3], belongs to class IB anti-arrhythmic agents [2] & works as a Non-selective voltage-gated sodium channel blocker. It has some ommon side effects chest discomfort, abdominal pain, nausea, headache, drowsiness. By delaying the recovery from inactivation of sodium channels, it prologs the refractory period for phase 0 of cardiac acton potential [4].

To develop new simple, sensitive, accurate and economical analytical method for theestimation of Mexiletine.

To validate the proposed method in accordance with USP and ICH guidelines for the intended analytical application.

MATERIALS & METHODS

HPLC, pH meter, Weighing machine, Volumetric flasks Borosil, Pipettes and Burette, Beakers, Digital ultra sonicator, Mexiletine (Pure), Water and Methanol for HPLC, Acetonitrile for HPLC.

HPLC METHOD DEVELOPMENT

Preparation of standard solution

To 10 mg of Mexiletine into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and remove air completely and make volume up to the mark with Methanol. Further pipette 0.6ml of the above Mexiletine stock solutions into a 10ml volumetric flask & dilute up to the mark with Methanol.

Procedure

Chromatograms were recorded, with the injection of the samples by changing the chromatographic conditions and noted the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Mobile Phase Optimization

Initially the mobile phase tried was Acetonitrile: Water and Methanol: Water with varying proportions. Finally, the mobile phase was optimized to Methanol: Water in proportion 50:50 v/v respectively.

Optimization of Column

The method was performed with various columns like C18 column, X- bridge column, Xterra, and C18 column. Symmetry C18 5µm (4.6×150mm) 5 µ was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

OPTIMIZED CHROMATOGRAPHIC CONDITIONS

Instrument used: Waters HPLC with auto sampler and PDA 486 Detector.
Column: Symmetry C18 5µm (4.6×150mm, 5 µ)
Column temperature: 40ºC
Wavelength: 270nm
Mobile phase ratio: Methanol: Water (50:50%v/v)
Flow rate: 0.9ml/min
Injection volume: 10 µl
Run time: 6min

VALIDATION

Preparation of mobile phase

Preparation of mobile phase

Accurately measured 500 ml (50%) of HPLC Water and 500 ml of Methanol (50%) were mixed and degassed by sonication for 10 minutes and then filtered through 0.45 µ filterunder vacuum filtration.

Diluent Preparation

The Mobile phase was used as the diluent.

VALIDATION PARAMETERS

System suitability

10 mg of Mexiletine working standard was accurately weighed and transferred into a 10ml of clean dry volumetric flasks & 7mL of Diluents & kept for sonication to dissolve it completely and made volume up to the mark with the same solvent. (Stock solution)

0.6ml of the above Mexiletine was pippeted out from stock solution into a 10ml volumetric flask and diluted up to the mark with diluent.

Procedure

The standard solution was injected for five times and measured the area for all five injections inHPLC. The %RSD for the area of five replicate injections was found to be within the specifiedlimits.

SPECIFICITY STUDY OF DRUG

Preparation of Standard Solution

Transfer 10 mg of Mexiletine was accurately weighed from working standard into a 10ml of clean dry volumetric flasks & added about 7ml of Diluents and sonicated to dissolve it completely and volume made up to the mark with the same solvent. (Stock solution)

Further 0.6ml of the above Mexiletine stock solution was pippeted into a 10ml volumetric flask and diluted up to the mark with diluents.

Preparation of Sample Solution

An average weight of the powder was taken and weighed 10 mg equivalent weight of Mexiletinesample into a 10mL clean dry volumetric flask which was take with 7mL of Diluent and sonicated to dissolve it completely and made volume up to the mark with the same solvent. Further 0.6ml of Mexiletine from above stock solution was pippeted out into a 10ml volumetric flask and diluted up to the mark with diluent.

Procedure

Inject the three replicate injections of standard and sample solutions and calculate the assay by using formula:

%ASSAY =

Sample area Weight of standard Dilution of sample Purity Weight of tablet

_____ × _____ × _____×_____×_____×100

Standard area Dilution of standard Weight of sample 100 Label claim

PREPARATION OF DRUG SOLUTIONS FOR LINEARITY

About 10 mg of Mexiletine was weighed and transferred as working standard into a 10ml of clean dry volumetric flasks & about 7ml of Diluents were added and sonicated to dissolve it completely and made volume up to the mark with the same solvent. (Stock solution)

Preparation of Level – I (20ppm of Mexiletine); Level – II (40ppm of Mexiletine); Level – III (60ppm of Mexiletine); Level – IV (80ppm of Mexiletine); Level – V (100ppm of Mexiletine)

Here,0.2ml, 0.4ml, 0.6ml, 0.8ml, 1.0mlof stock solution was taken in to 10ml of volumetric flask and made volume up tomark with diluent

Procedure

In the chromatographic system,at each level injection was done & peak area was measured. A graph of peak area versus concentration was plotted (on X-axis concentration and on Y-axis Peak area) and correlation coefficient was calculated.

PRECISION

Repeatability

Preparation of Mexiletine Product Solution for Precision

Accurately weigh and transfer 10 mg of Mexiletine working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and made volume up to the mark with the same solvent. (Stock solution)

Further pipette about 0.6ml of the above Mexiletine stock solutions into a 10ml volumetric flask and diluted up to the mark with diluents. The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Intermediate precision

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precisionwas performed on different days by maintaining same conditions.

Procedure

Analyst 1 & 2

The standard solution was injected for Six times and measured the area for all Six injections in HPLC. The %RSD for the area of Six replicate injections was found to be within the specified limits.

Accuracy

For preparation of 50%,100%,150% Standard stock solution

10 mg of Mexiletine was accurately weighed and transferred as working standard into a 10ml of clean dry volumetric flasks & about 7mL of Diluents were added and sonicated to dissolve it completely and volume was made up to the mark with the same solvent. (Stock solution)

Further 0.3ml, 0.6, 0.9ml of the above Mexiletine stock solution was pippeted into a 10ml volumetric flask and diluted up to the mark with diluents.

Procedure

Three replicate injections of individual concentrations (50%, 100%, 150%) were made under the optimized conditions. Recorded the chromatograms and measured the peak responses. The amount found, amount added for Mexiletine, individual recovery and mean recovery values were calculated.

ROBUSTNESS

The analysis was performed in different conditions to find the variability of test results. The following conditions are checked for variation of results.

For preparation of Standard solution

10 mg of Mexiletine as working standard was accurately weighed & added into a 10ml of clean dry volumetric flasks & about 7mL of Diluents was added and sonicated to dissolve it completely and made volume up to the mark with the same solvent. (Stock solution). Further 0.6ml of the above Mexiletine stock solution was pippeted into a 10ml volumetric flask and diluted up to the mark.

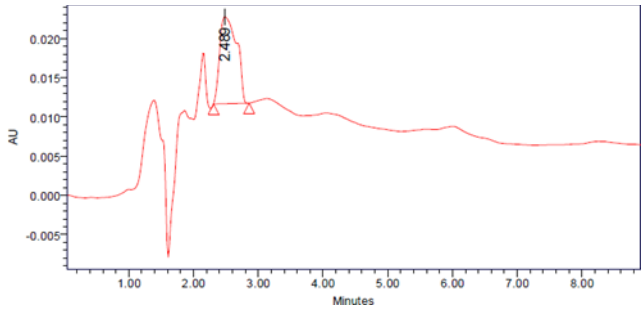
Effect of Variation of flow conditions

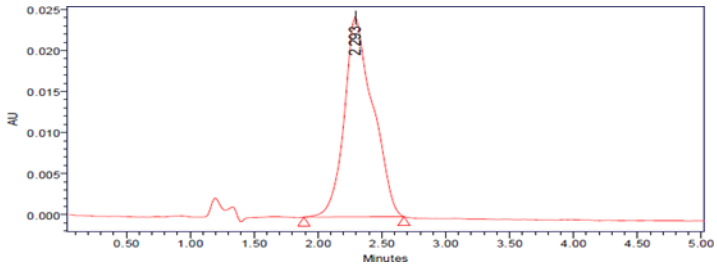
The sample was analyzed at 0.8ml/min and 1.0ml/min instead of 0.9ml/min, remaining conditions were same. 10µl of the above sample was injected and chromatograms were recorded.

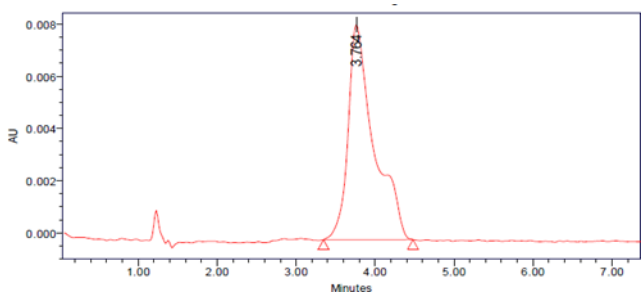
RESULTS

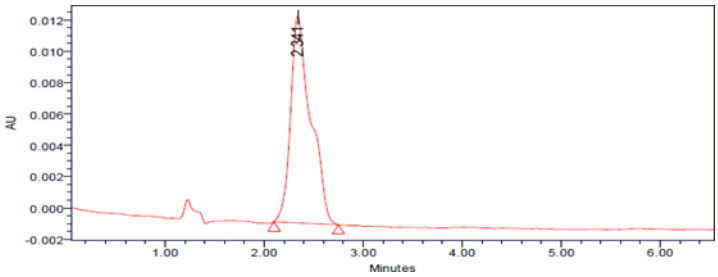
Trails	Column	Column temperature	Wavelength	Mobile phase ratio	Flow rate	Injection volume	Run time
Trail I	X terra C18 (4.6×250mm, 5µm)	30ºC	270nm	Water: Methanol (10:90%v/v)	1.0 ml/min	10µl	9min
Trail II	X terra C18(4.6×250	35ºC	270nm	Methanol: Water (65:35%v/v)	1.0 ml/min	10 µl	8min

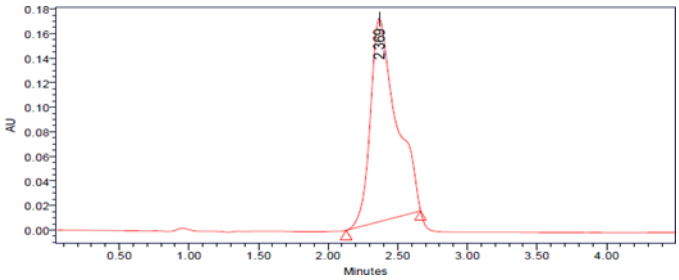
	mm, 5µm)						
Trail III	X bridge C18 (4.6×150mm, 5µm)	35ºC	270nm	Water: (40:60%v/v)	1.0 ml/min	10 µl	7min
Trail IV	ODS C18 (4.6×150mm, 5µm)	35ºC	270nm	Water: ACN	0.8ml/min	10 µl	5min
Trail V	X bridge C18 (4.6×150mm, 5µm)	0ºC	270nm	Water: ACN (50:50%v/v)	0.8ml/min	10 µl	5min







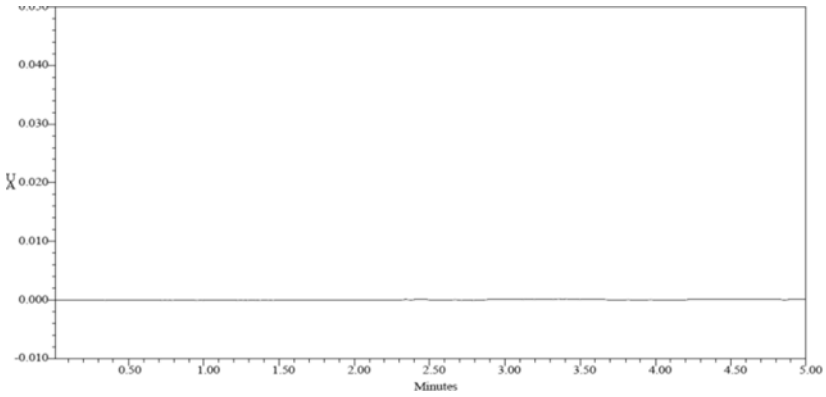




Trail No	Peak name	Rt	Area	Height	USP tailing	USP Plate count
I	Mexiletine	2.489	11102	18910	1.3	985
II	Mexiletine	3.764	8256	29823	1.3	569
III	Mexiletine	2.341	186704	13255	1.4	5011
IV	Mexiletine	2.293	379132	24376	1.4	5112
V	Mexiletine	2.369	2124443	166186	1.4	5211

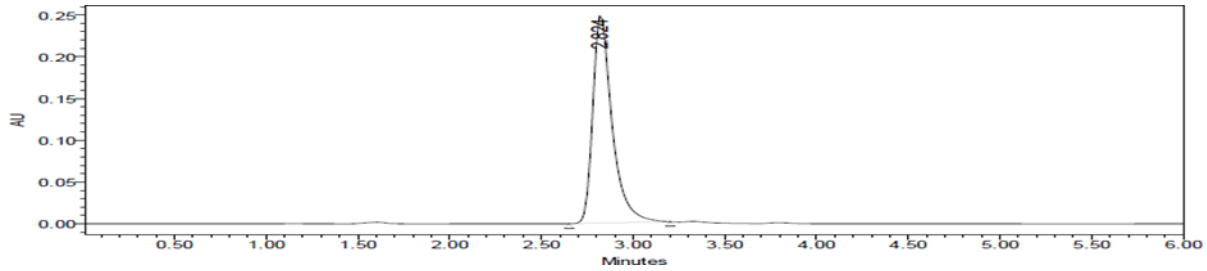
VALIDATION

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Mexiletine	2.824	1819564	249911	3965	1.4
2	Mexiletine	2.825	1822439	252600	3998	1.4
3	Mexiletine	2.827	1819738	255482	4015	1.4
4	Mexiletine	2.822	1816041	249241	3975	1.4
5	Mexiletine	2.830	1812710	245336	4215	1.4
Mean			1818098			
Std. Dev.			3773.09			
% RSD			0.2			

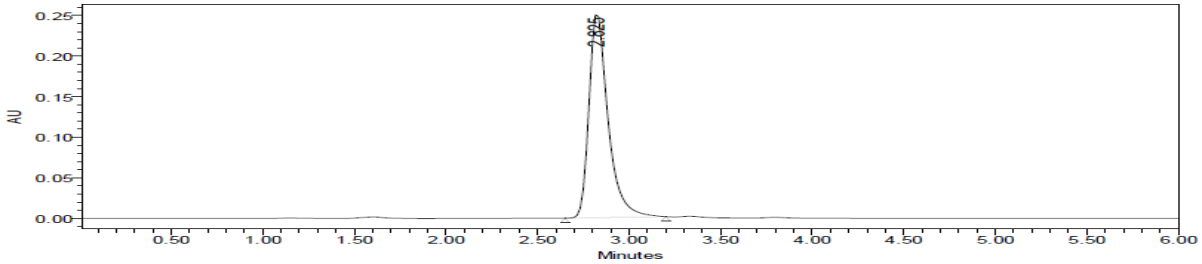


Chromatogram showing blank (mobile phase preparation)

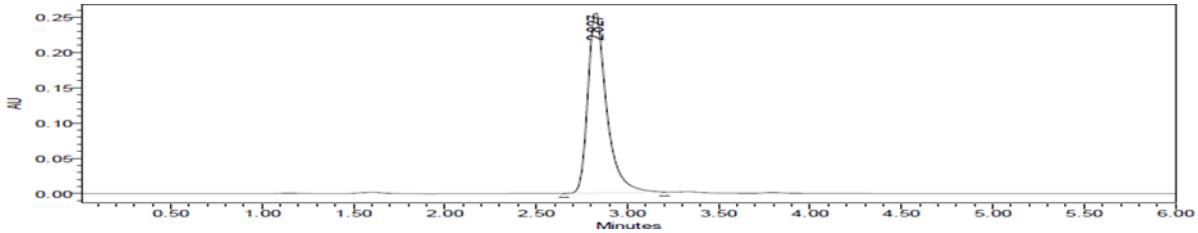
System suitability



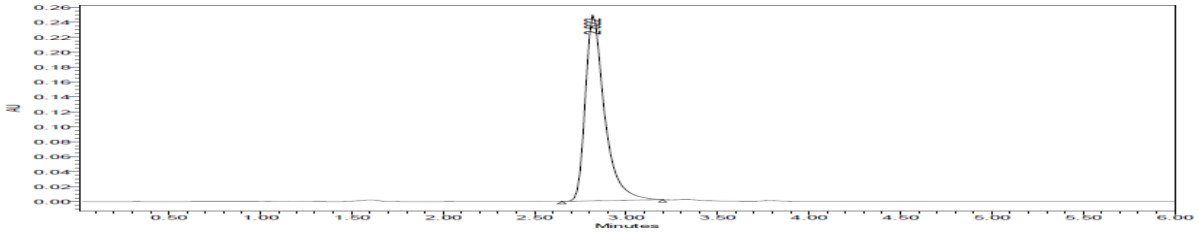
Chromatogram showing injection -1



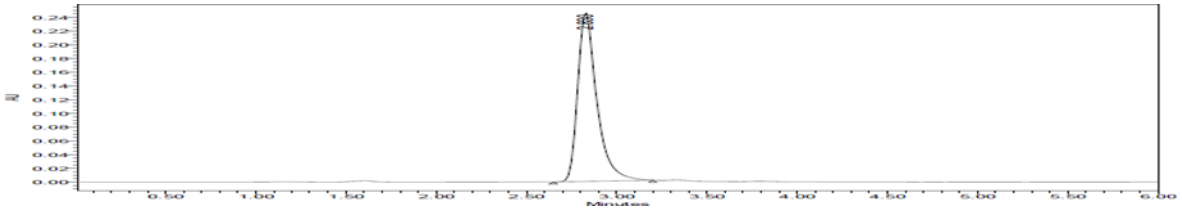
Chromatogram showing injection -2



Chromatogram showing injection -3

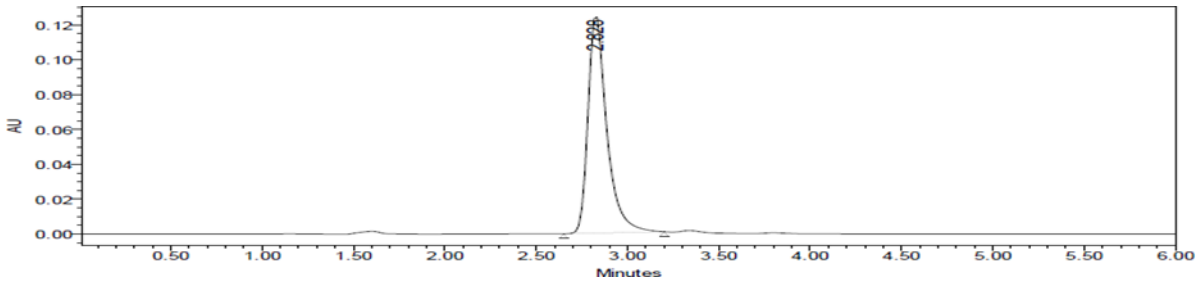


Chromatogram showing injection -4

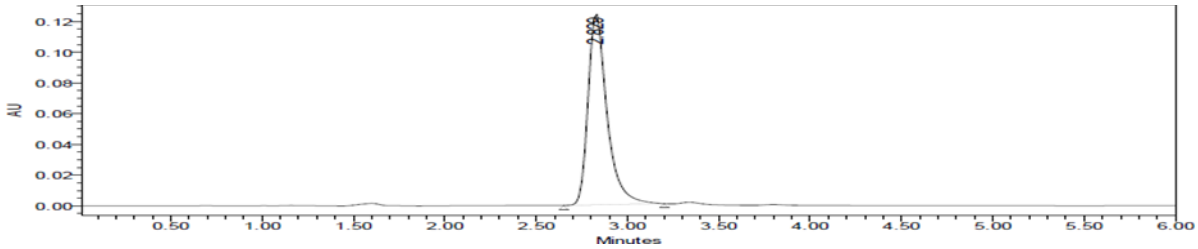


Chromatogram showing injection -5

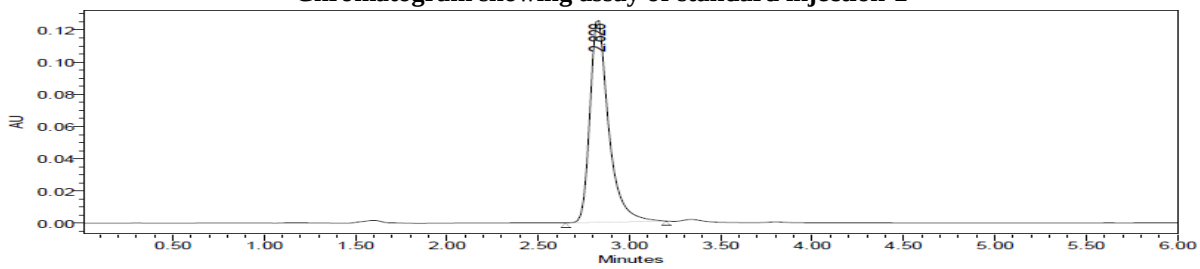
SPECIFICITY



Chromatogram showing assay of standard injection-1

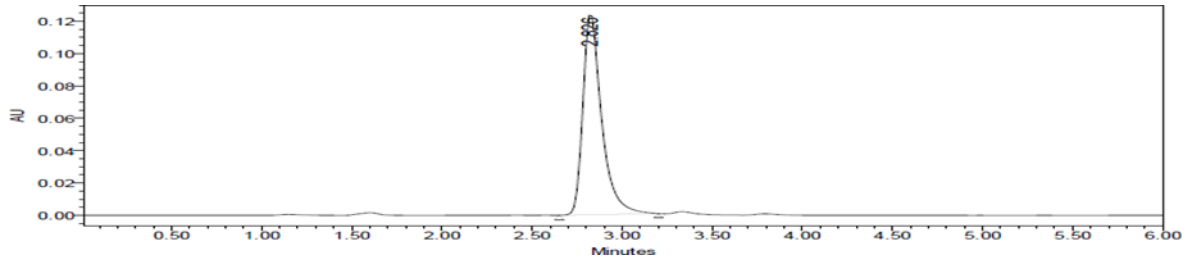


Chromatogram showing assay of standard injection-2

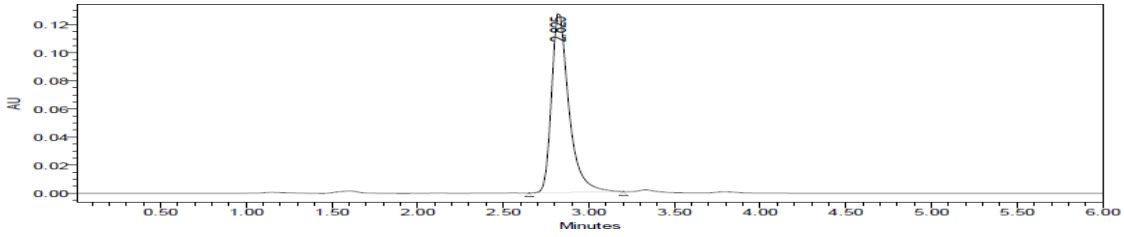


Chromatogram showing assay of standard injection-3

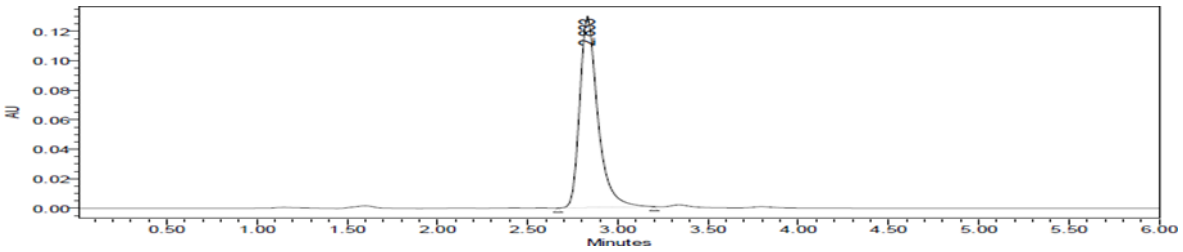
ASSAY (SAMPLE)



Chromatogram showing assay of sample injection-1



Chromatogram showing assay of sample injection-2



Chromatogram showing assay of sampleinjection-3

%ASSAY =

$$\frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weightofstandard}}{\text{Dilutionofstandard}} \times \frac{\text{Dilution ofsample}}{\text{Weightofsample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight oftablet}}{\text{Label claim}} \times 100$$

The % purity of Mexiletine in pharmaceutical dosage form was found to be100.6%.

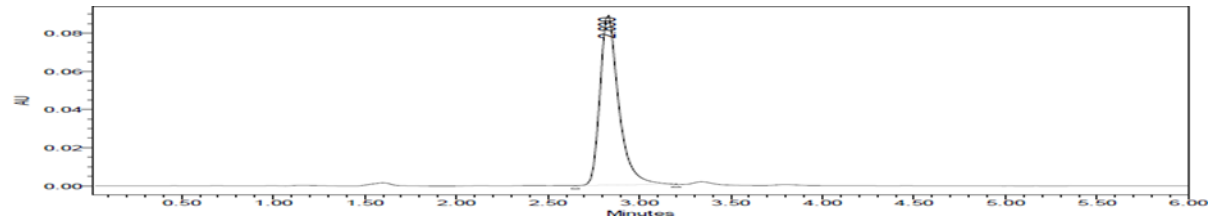
Peak results for assay standard

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Mexiletine	2.828892717	1242361.4			3922.9	1
2	Mexiletine	2.829899298	1240291.4			3883.2	2
3	Mexiletine	2.828891366	1255251.4			4023.9	3

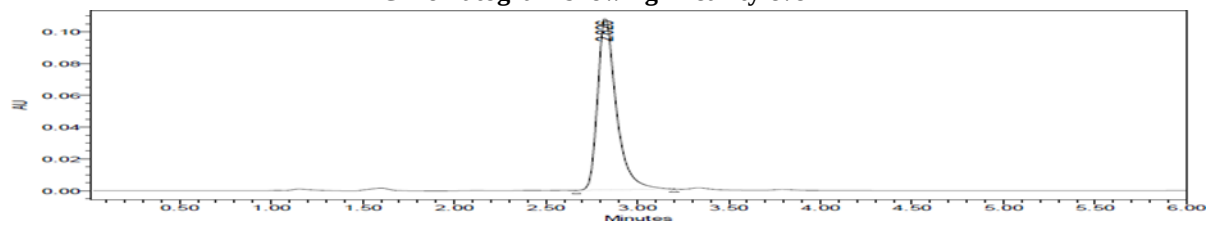
Peak results for Assay sample

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Mexiletine	2.826896	838124	8691.4		3928.6	1
2	Mexiletine	2.825898	2921286	871.4		4568.8	2
3	Mexiletine	2.833901	4961292	001.4		5693.0	3

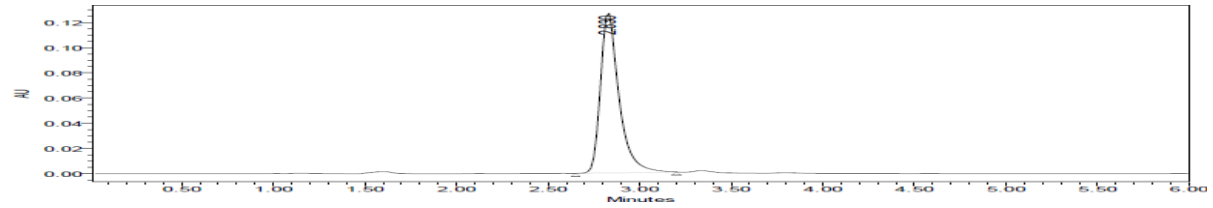
LINEARITY



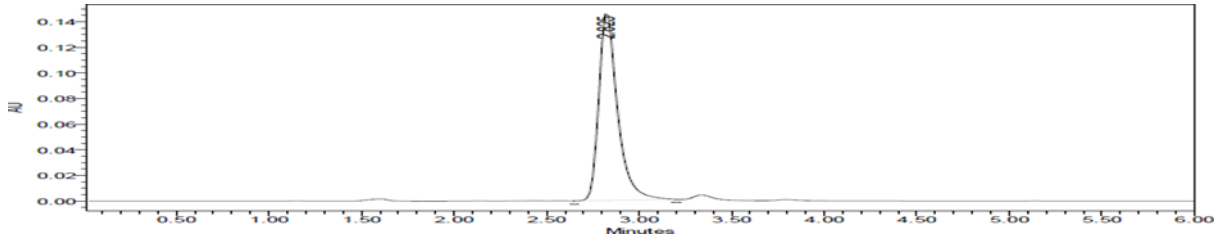
Chromatogram showing linearitylevel-1



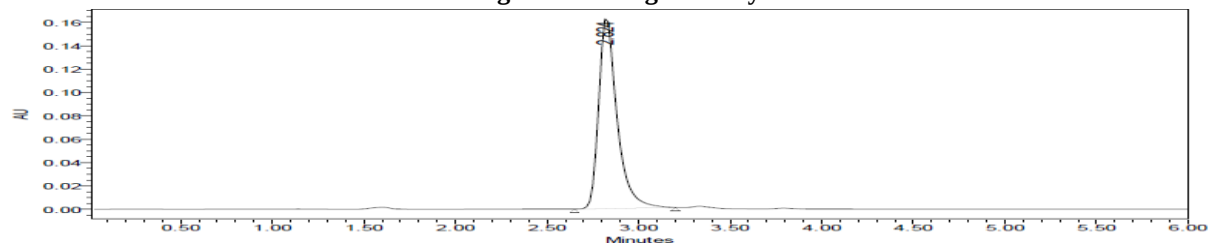
Chromatogram showing linearitylevel-2



Chromatogram showing linearitylevel-3



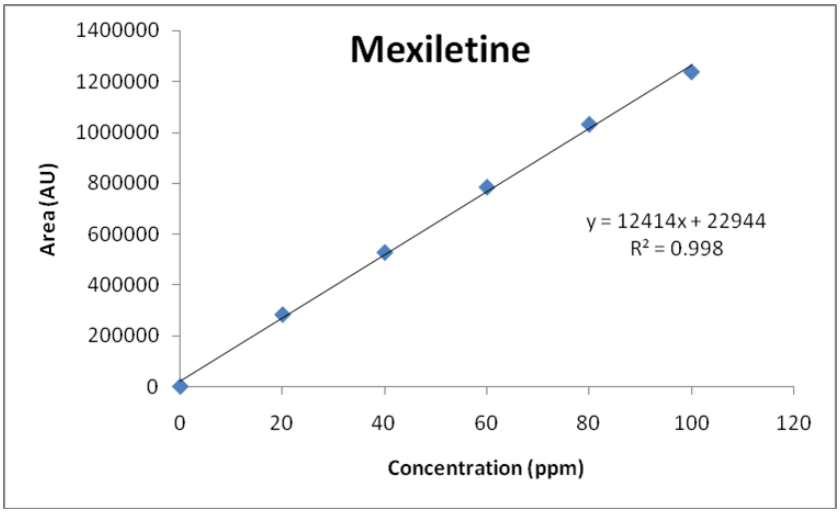
Chromatogram showing linearitylevel-4



Chromatogram showing linearitylevel-5

CHROMATOGRAPHIC DATA FOR LINEARITY STUDY

Concentration Level (%)	Concentration	Average
	□g/ml	Peak Area
33	20	282417
66	40	526995
100	60	783937
166	80	1031164
133	100	1237297



RESULTS OF REPEATABILITY FOR MEXILETINE

S. No	Peak name	Retention time	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Mexiletine	2.824	894562	128135	3981	1.3
2	Mexiletine	2.827	896754	129139	4213	1.4
3	Mexiletine	2.833	893627	132891	4562	1.4
4	Mexiletine	2.833	893750	129914	4562	1.4
5	Mexiletine	2.836	892682	130515	4610	1.4
Mean			894275			
Std.dev			1537.936			
%RSD			0.171976			

Results of Intermediate precision for Mexiletine

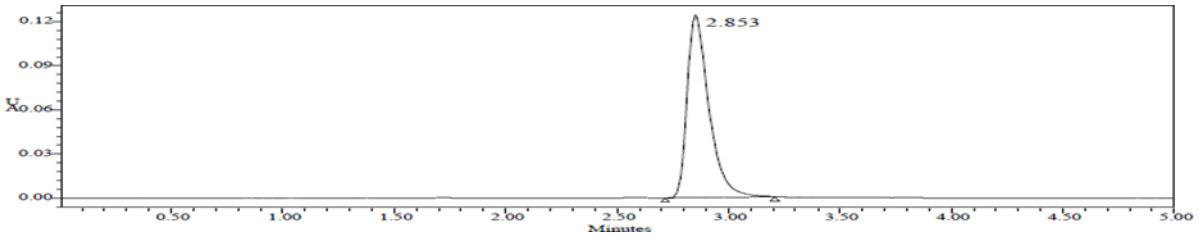
S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Mexiletine	2.823	895311	125747	4510	1.4
2	Mexiletine	2.827	896783	122578	4002	1.4
3	Mexiletine	2.828	895237	124365	4235	1.4
4	Mexiletine	2.828	894206	124057	4235	1.4
5	Mexiletine	2.825	895085	125410	4015	1.4
6	Mexiletine	2.822	896041	129241	3998	1.3
Mean			895443.8			
Std. Dev.			879.931			
% RSD			0.09			

Results of Intermediate precision Analyst 2 for Mexiletine

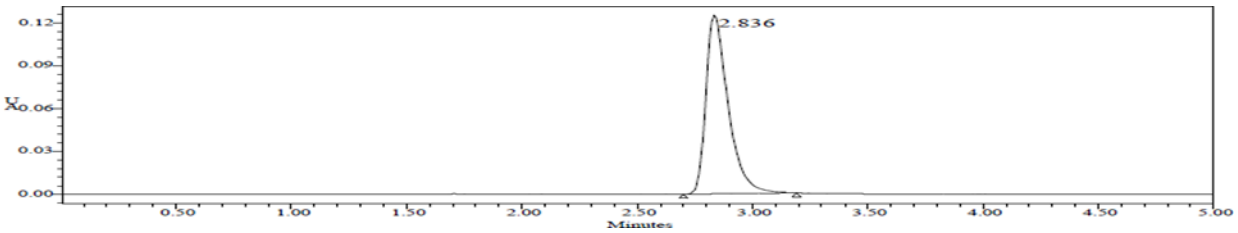
S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Mexiletine	2.833	893627	132891	4986	1.4
2	Mexiletine	2.836	892682	130515	5123	1.4
3	Mexiletine	2.827	896754	129139	4081	1.4
4	Mexiletine	2.827	896754	129139	4150	1.4
5	Mexiletine	2.823	895311	125747	4051	1.3
6	Mexiletine	2.827	896783	122578	4150	1.4
Mean			895318.5			
Std. Dev.			1793.234			
% RSD			0.2			

ACCURACY

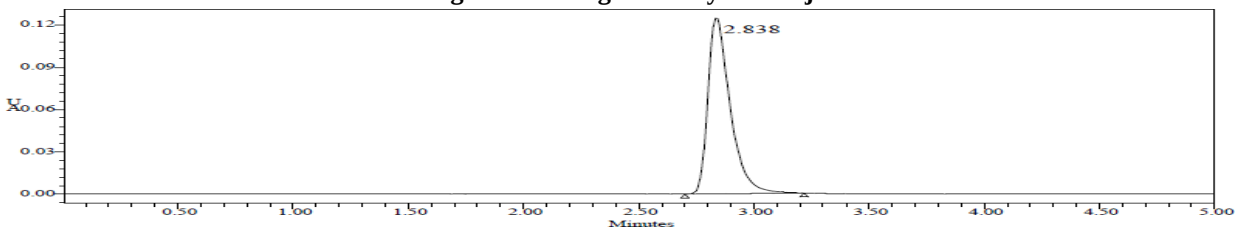
Accuracy at different concentrations (50%, 100%, and 150%) were prepared and the % recovery was calculated.



Chromatogram showing accuracy-50%injection-1



Chromatogram showing accuracy-50%injection-2

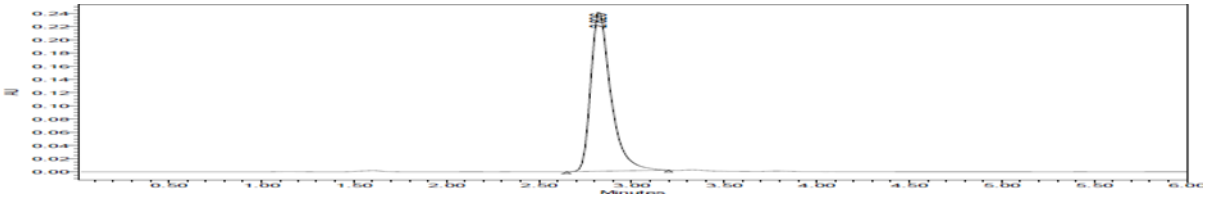


Chromatogram showing accuracy-50% injection-3

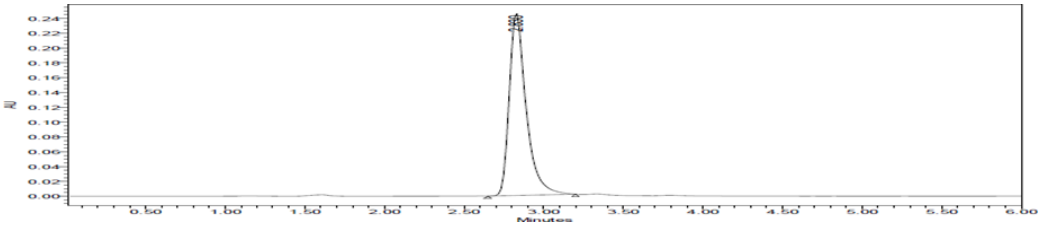
Results of Accuracy for concentration-50%

S.No	Name	RT	Area	HeightUSP Tailing	USP Plate Count	Injection
1	Mexiletine	2.836	390550	4176311.4	5123	1
2	Mexiletine	2.838	398022	4177171.4	4987	2
3	Mexiletine	2.853	402044	4148311.3	4996	3

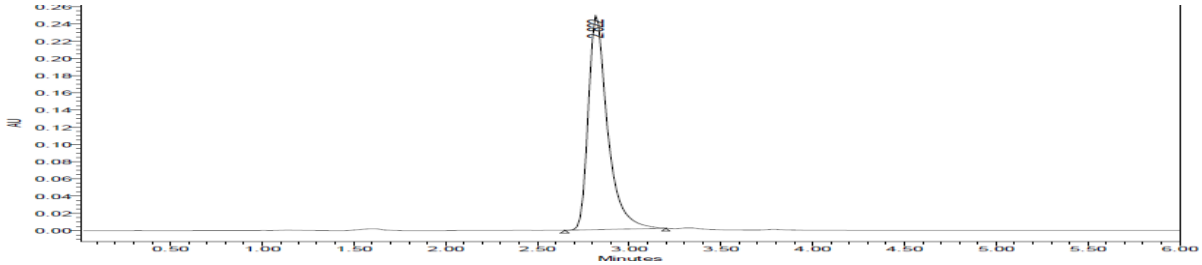
Accuracy100%:



Chromatogram showing accuracy-100%injection-1



Chromatogram showing accuracy-100%injection-2

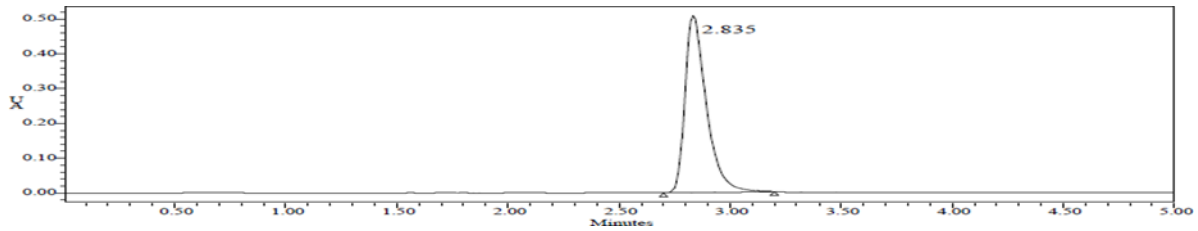


Chromatogram showing accuracy-100% injection-3

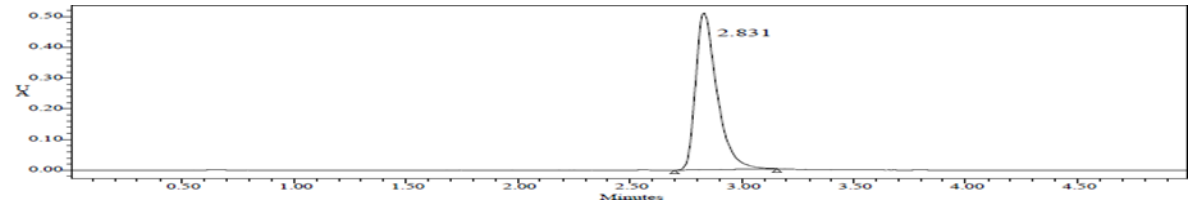
Results of Accuracy for concentration-100%

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Mexiletine	2.826	7682892	437901.4		5210	1
2	Mexiletine	2.830	7552492	453361.4		4989	2
3	Mexiletine	2.822	7770222	492411.4		5031	3

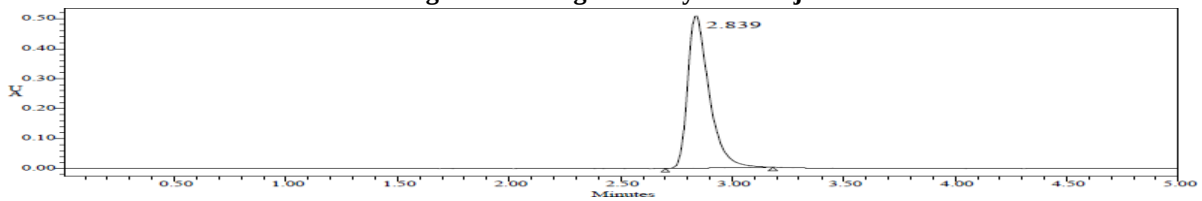
Accuracy150%



Chromatogram showing accuracy-150%injection-1



Chromatogram showing accuracy-150%injection-2



Chromatogram showing accuracy-150% injection-3

Results of Accuracy for concentration-150%

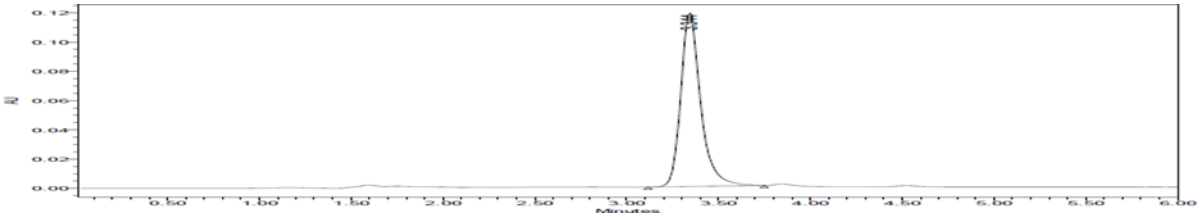
S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Mexiletine	2.831	12103535098321.4			4987	1
2	Mexiletine	2.835	1101475083981.4			5364	2
3	Mexiletine	2.839	1109405082161.4			5412	3

The accuracy results for Mexiletine

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	406872	30	29.8	99.3	
100%	766853.360		59.9	99.8	
150%	1143813	90	89.6	99.5	
					99.5%

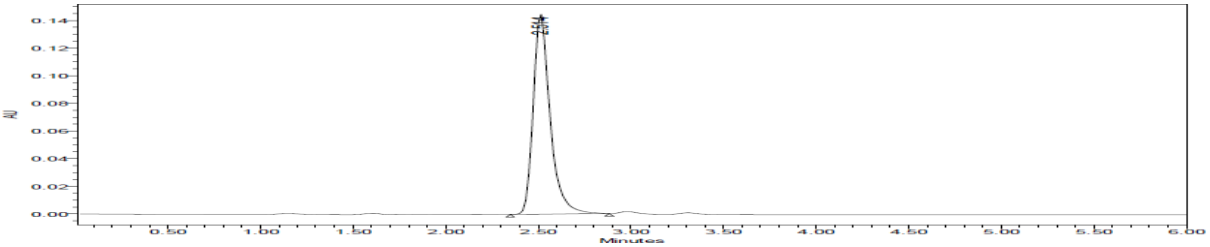
Robustness

Variation of mobile phase organic composition



(Chromatogram showing less organic composition)

Variation of mobile phase organic composition



(Chromatogram showing more organic composition)

RESULTS FOR ROBUSTNESS

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 0.9mL/min	1817227	2.826	4531	1.4
Less Flow rate of 0.8mL/min	1005760	3.13	4921.2	1.4
More Flow rate of 1.0mL/min	819776	2.589	4493.3	1.4
More Organic phase	922032	2.514	3834.7	1.3
Less Organic phase	893128	3.344	5032.7	1.3

CONCLUSION

- In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Mexiletine in bulk drug and pharmaceutical dosage forms.
- This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps.

- Mexiletine was freely soluble in ethanol, methanol and sparingly soluble in water.
- Methanol: Water was chosen as the mobile phase. The solvent system used in this method was economical.
- The %RSD values were within 2 and the method was found to be precise.
- The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods.
- This method can be used for the routine determination of Mexiletine in bulk drug and in Pharmaceutical dosage forms.

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