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ANALYTICAL ESTIMATION AND VALIDATION OF LAMOTRIGINE BY NEW HPLC METHOD

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Abstract
A simple, rapid, reproducible and precise method was developed for the quantitative determination of lamotrigine in its pure form as well as in pharmaceutical dosage forms. The method is based on high performance liquid chromatography. Alliance Waters e2695 Separations Module with Waters 2774 pump HPLC system equipped with autosampler, ultraviolet detector, and Waters Atlantis T3 C-18 5µm, 4.6 X 150 mm column were used for the quantification of the drug. Separation was carried out at a flow rate of 0.7 mL/min. of mobile phase (acetonitrile and ethanol) and the detection was carried out at a wavelength of 275 nm. The lamotrigine was well resolved on the stationary phase and the method was validated and shown to be linear for lamotrigine in the range of 5-25 µg/ml. The method was validated for Precision, Accuracy, LOD and LOQ were determined and the correlation coefficient for lamotrigine was found to be 0.9901 respectively. The developed method was found to be repeatable and reproducible; hence, it can be used as an alternative method in assay of the lamotrigine in any pharmaceutical industries.

Keywords: Lamotrigine, Method Development, Validation, HPLC

Introduction

Lamotrigine is an anti-epileptic and used to treat psychotic problems. It is referred to as dichlorophenyl triazine diamine. The drug balances mood and it is the first drug for treating bipolar illness after lithium. Because of its chemical composition, Lamotrigine has extremely few side effects as compared to other anticonvulsants, and it doesn't require blood monitoring.(Batool et al.,2022)

Lamotrigine prevents voltage-sensitive Na+ channels, which balances chemical messengers and alters release of glutamate and aspartate from presynaptic transmitters, according to in vitro pharmacological study.(Anantha et al.,2010)

Previously, many techniques based on HPLC7-19, LC-MS20-22, HPTLC23, and spectrophotometry 24-26 were published to measure Lamotrigine in formulations and relation to body fluids, both alone and in mutual with different medications and associated compounds. The current work describes a fast, precise and accurate Reverse-HPLC technique to determine the lamotrigine content of formulation dosage forms.(Staut et al.,2005)

Method Development of Lamotrigine

1. Preparation of mobile phase:

- To produce the mobile phase, 700 ml of acetonitrile and 300 ml of ethanol (7:3) were combined. The two solvents were thoroughly combined and allowed to degas in an ultrasonic water bath for 30 minutes. Using a 0.45 µm membrane filter, the final solutions was filtered.(Reddy et al.,2008)

2. Preparation of std. (stock) solution:

- In a flask (100 ml), 10 mg of precisely weighed lamotrigine (API) were dissolved in 70 ml solution, sonicated, and topped up to the necessary quantity to make lamotrigine (LMG) stock solution.
- The 1.5 ml of the solution described above is diluted by adding sufficient quantity of mobile phase up to the mark into a 10 ml volumetric flask to prepare the working standard solution of concentration of 15µg/ml.
- The std. (stock) solution was then sonicated in sonicator and filtered through 0.45µm filter.
- Following the same process, various dilute solutions were prepared for various ranges of concentration and then put into an autosampler vial for further analysis.(bhosle et al.,2011)

Chromatographic Conditions

At the flow rate of 0.7 ml/min, the column was filled by injecting it from the solvent reservoir. 30-minute equilibration period was given to the column before 20 µl of the standard was injected. At 275 nm, the components that had eluted from the column were detected.

Tests and Discussion

Specificity

Comparing the drug's chromatogram with the blank (mobile phase) chromatogram allowed for technique specificity to be determined. The working standard solution was added to a 20µl loop at a concentration of 15µg/ml, and then the chromatogram was recorded. It was discovered that the component's retention duration was 6.235 minutes.

S no.	Peak name	Retentime time	Area	Height
1	**Lamotrigine**	6.235	616423	148659

Table 1: Table of Retention Time of Lamotrigine

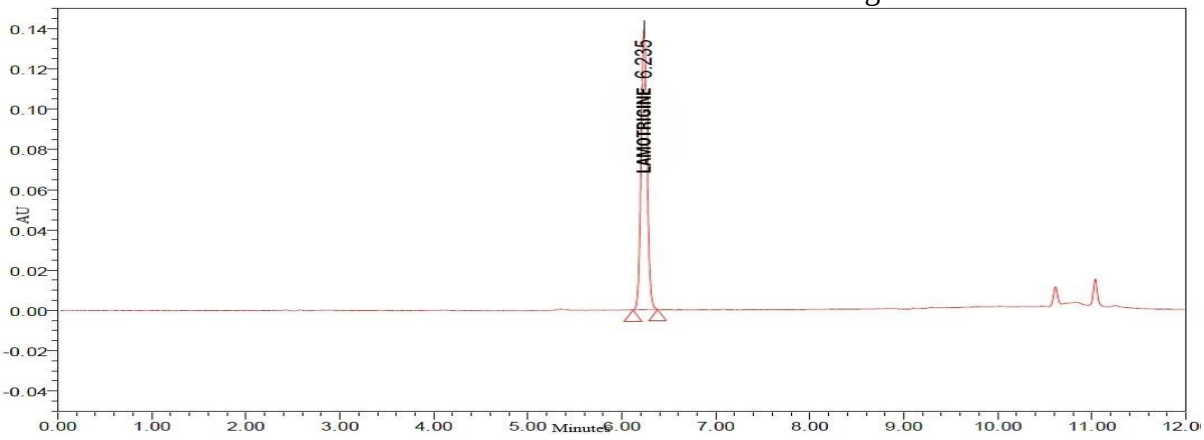

Figure 1: Chromotogram of Lamotrigine

Validation of the method

- **Precision of the method:**
- **Method Precision Study:** Five runs of 2 different concentrations (150 ppm and 200 ppm), were given on the same day and the valuation of correlation coefficient was calculated to determine intra-day precision.(Reddy et al.,2011)

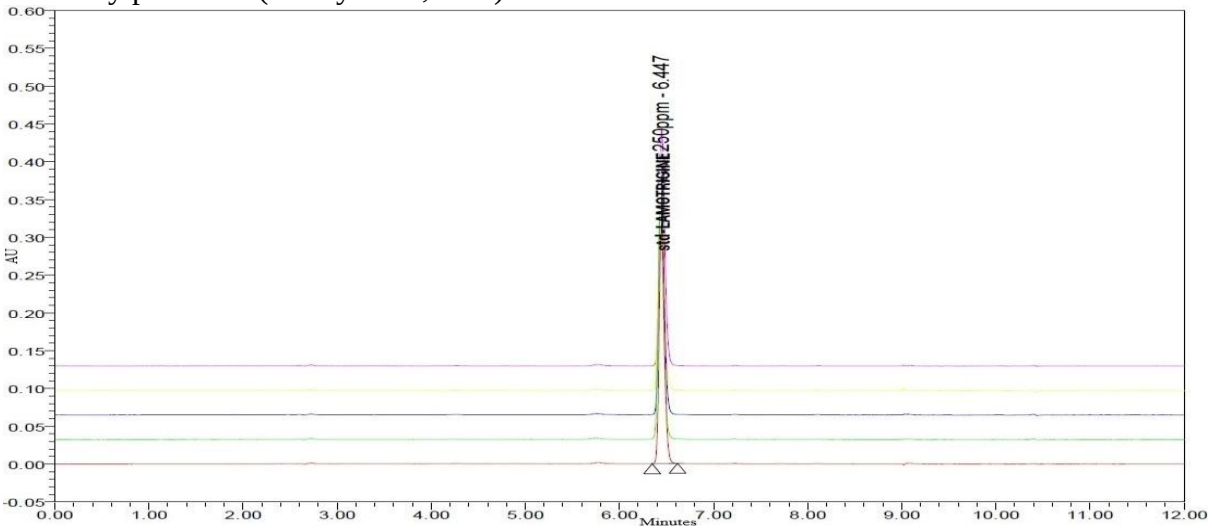

Figure 2: Chromatogram peak summary report of I to VI run of 250 ppm solution.

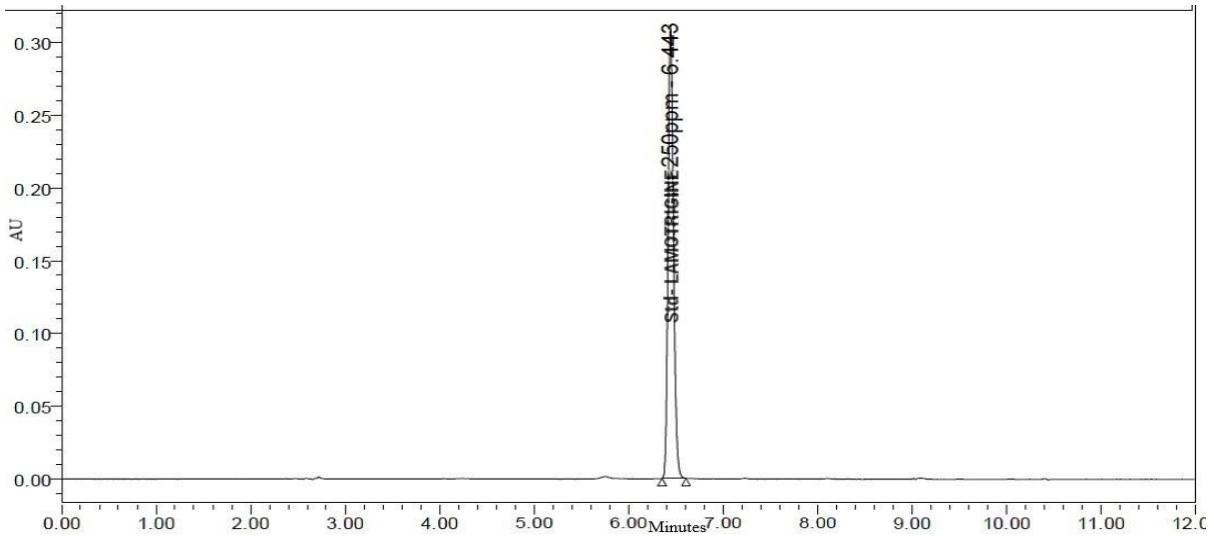

Figure 3: Chromatogram Peak summary report for interday precision study of 300 ppm

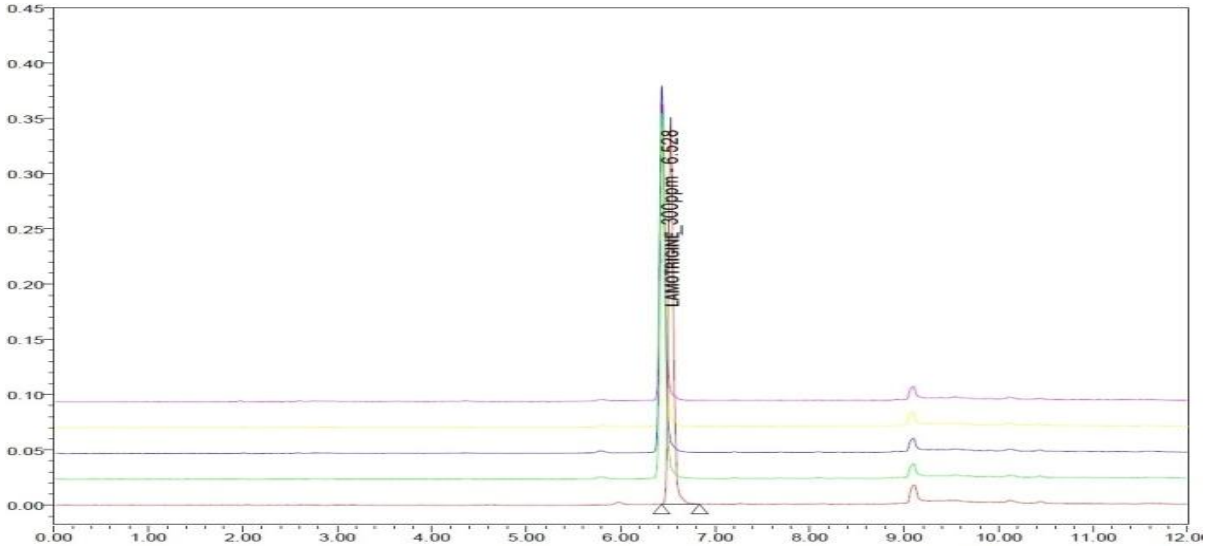

Figure 4: Summary report of run of 30.0 ppm solution for Chromatogram Peak.

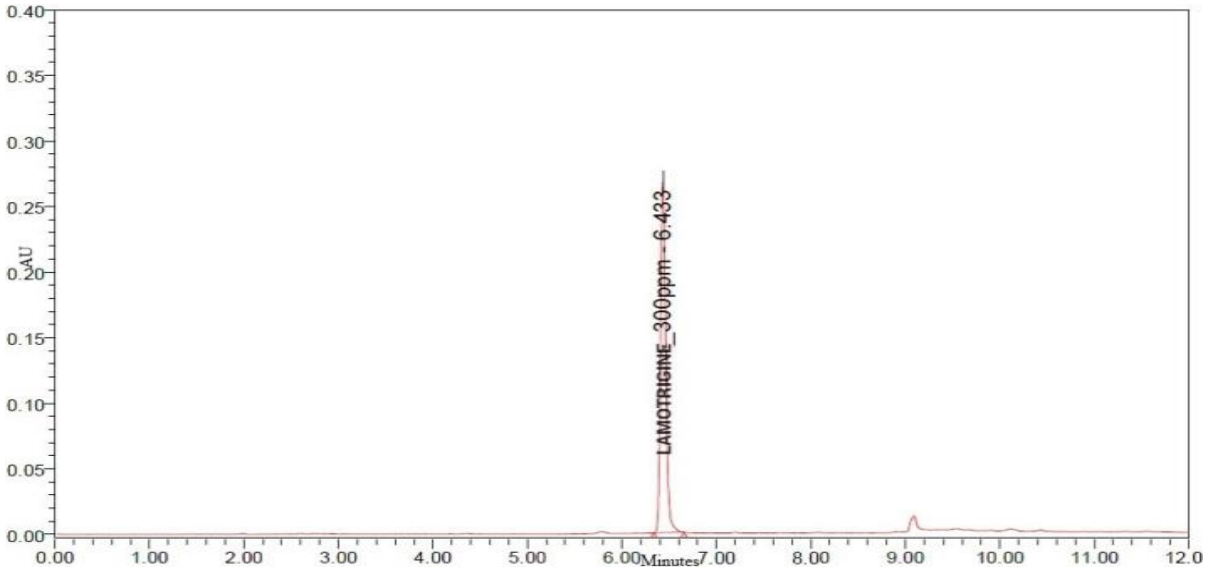

Figure 5: Summary report of VI run of 300 ppm solution of Chromatogram Peak.

Chromatogram Peak summary report for intraday precision study

S No. / RUN	INTRADAY AREA		INTERDAY AREA	
	250PPM	**300PPM**	**300PPM**	**250PPM**
FIRST	1243879	1286578	1029191	1241316
SECOND	1238983	1289609	1037285	1242744
THIRD	1245043	1283311	1043998	1243879
FOURTH	1242744	1288832	1037727	1238983
FIFTH	1241316	1290312	1047367	1245043
SIXTH	1238254	1291709	1038470	1240754
TOTAL	7450219	7730351	6234038	7452719
AVERAGE	1241703	1288391	1039006	1242120
SD	2698.81	3015.65	6261.71	2208.191
RSD	0.21	0.23	0.60	0.177

Table 2: Precision Data

- **Linearity of Method**:

Five different concentration of the drug were prepared for linearity studies. Response was measured as peak area. The calibration curve was obtained by plotting peak area (abs*s) against concentration (µg/ml).

Table 3: Linearity table showing Retention time, USP Plate count, Area and USP Tailing

LAMOTRIGINE	RUN	RT	AVE. RT	AREA	AVE. AREA	USP PLATE COUNT	AVE. PLATE COUNT	USP TAILING	AVE. TAILING
250PPM	1st RUN	6.447	6.444	851997	850441	68280	68551	1.202	1.197
	2nd RUN	6.442		848886		68822		1.193	
300PPM	1st RUN	6.528	6.484	1028832	1026071	72036	68503	1.227	1.236
	2nd RUN	6.440		1023311		64970		1.245	
350PPM	1st RUN	6.448	6.456	1108684	1104715	66999	68244	1.227	1.218
	2nd RUN	6.465		1100747		69489		1.210	
400PPM	1st RUN	6.482	6.498	1294803	1297620	69597	70753	1.237	1.231
	2nd RUN	6.514		1300437		71909		1.226	
450PPM	1st RUN	6.532	6.539	1429425	1432081	69997	70710	1.239	1.239
	2nd RUN	6.546		1434737		71423		1.239	

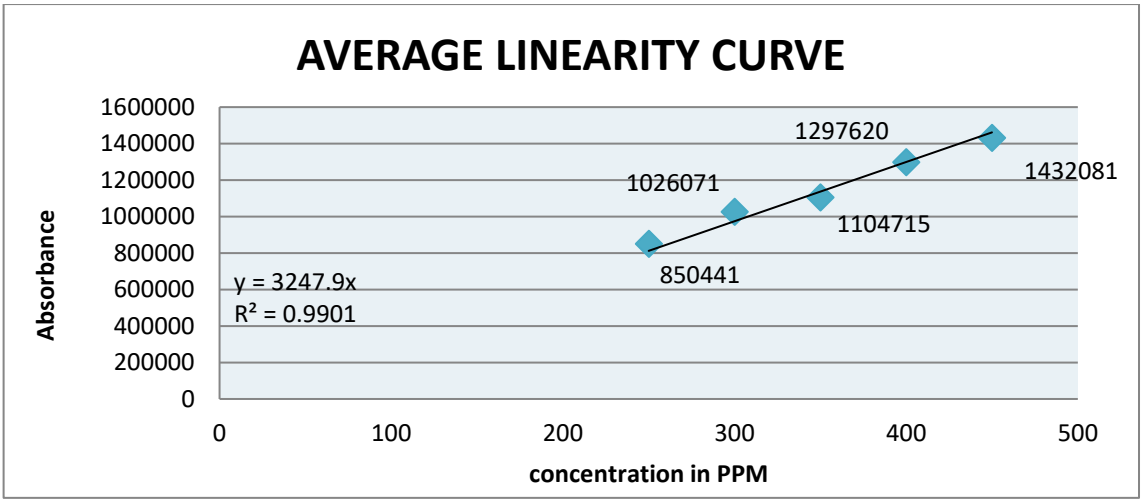

Figure 6: Average Linearity Curve

Concentration in PPM	Average Area	R^2
250.0	850441	0.9901
300.0	1026071	
350.0	1104715	
400.0	1297620	
450.0	1432081	

Table 4: Linearity data

- **Accuracy of the Method**

Accuracy

To assess accuracy, we looked at the percentage recovery for three separate Lamotrigine samples that were prepared: one was a control sample with a 300 ppm concentration; another was a spiked sample with 2 µg stock solution of Lamotrigine (Emami et al.,2006). Each was run three times, and the percentage recovery of the spiked sample and control sample was determined using the following formula:

$$\% \text{ Recovery} = \frac{\text{Theoretical amount}}{\text{Calculated amount}} \times 100$$

	Area				% Recovery		
	Standard sample	**Control Sample**	**80% Spike sample**	**120% Spike sample**	**Control Sample**	**80% Spike sample**	**120% Spike sample**
1st run	1243879	1037727	911304	1360993	119%	136%	91.3%
2nd run	1238983	1038470	908366	1353538	119.3%	136.3%	91.5%
3rd run	1245043	1047367	905783	1352107	118.8%	137.4%	92.0%
Mean=	1242635	1041188	908484	1355546	119%	136.56%	91.6%
S.D.=	2625.71	4379.72	7953.522	3895.66	0.2054	0.6018%	0.2943%
% RSD=	0.211	0.420	0.2482	0.2873	0.172	0.440%	0.3213%

Table 5: standard, control and spiked solution area and % recovery study

System suitability parameters

Capacity factor, resolution, tailing factor, retention volume, theoretical plate, asymmetry factor and the plate height of the peaks are determined for system suitability parameters.

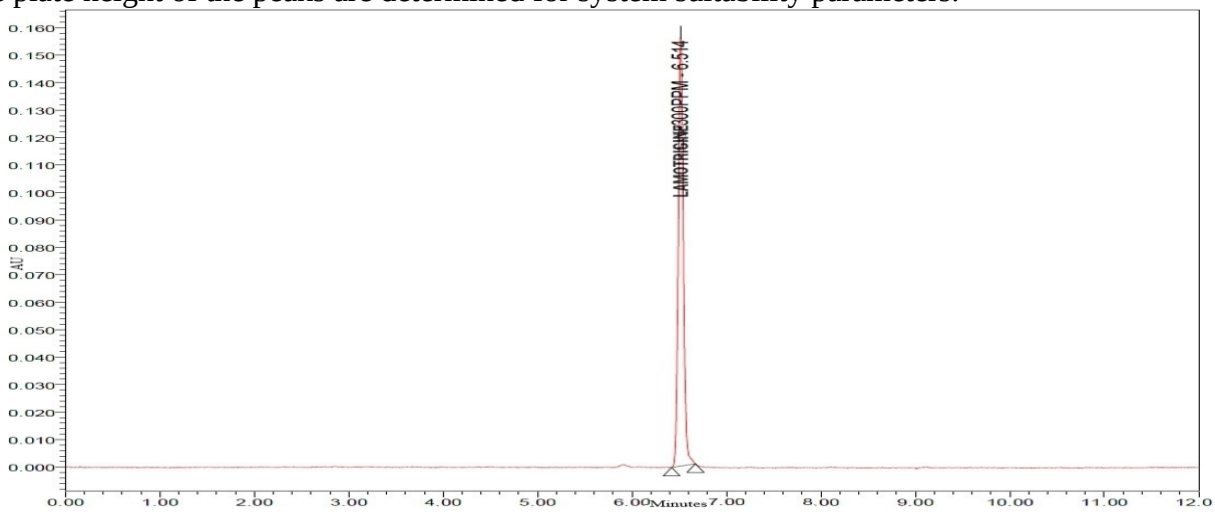

Figure 7: Chromatogram of standard sample

PARAMETERS	LAMOTRIGINE	PARAMETERS	LAMOTRIGINE
Retention Time (min)	6.514	Tailing factor	1.09
Capacity Factor (k)	5.5142	Resolution (Rs)	0.00
Theoretical Plates	74086	Separation	0.00

Table 6: System Suitability Parameters of Lamotrigine

Limit of detection (LOD) Data

By analysing samples with known concentrations of the analyte and identifying the minimum quantity at which the analyte may be consistently detected yield the limit of detection.(Gondhale et al.,2023)

The LOD solution had the concentration of 5ppm.

Following is the computation of the LOD:

$$LOD = \frac{3.3 \text{ X } 1515.15}{1000}$$

$$= 5.0$$

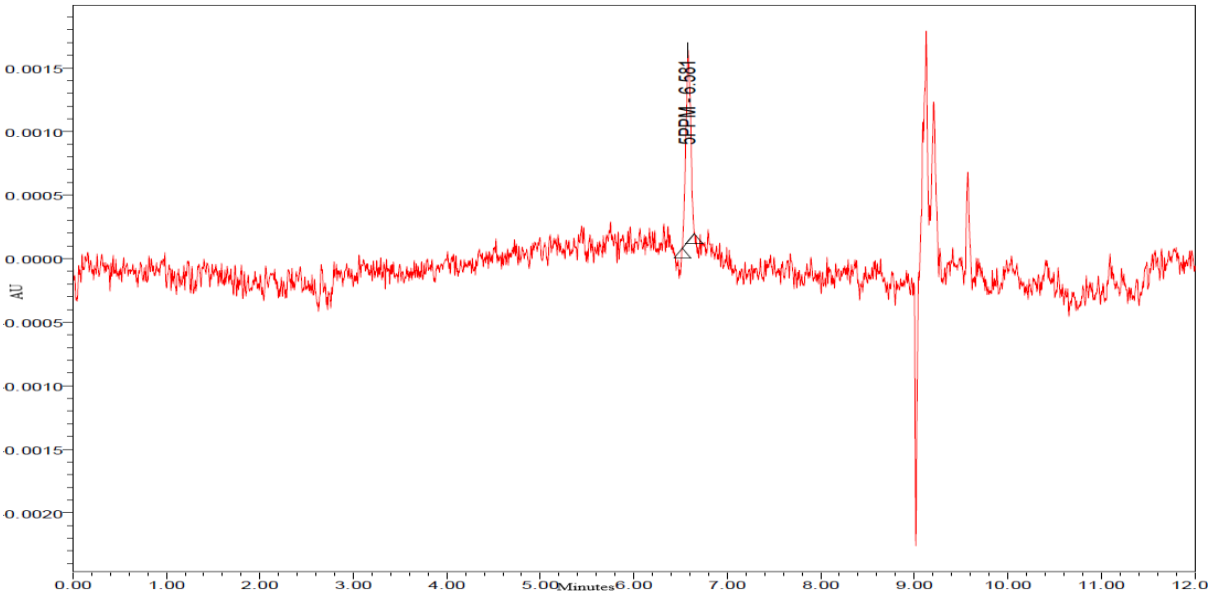

Figure 8: Chromatogram of LOD solution of Lamotrigine

Limit of quantization (LOQ) Data

By analysing the known concentrations of the analyte samples and identifying the minimum quantity at which the analyte may be quantified with reliable accuracy and precision yield the limit of qualification(Gondhale et al.,2023). Following is the computation of the limit of quantization:

LOQ = 10 (σ /S)

Where "σ"signifies the average standard deviation of the response and "S"is the slope of the calibration curve.

The concentration of LOQ solution was found to be 7.7 ppm.

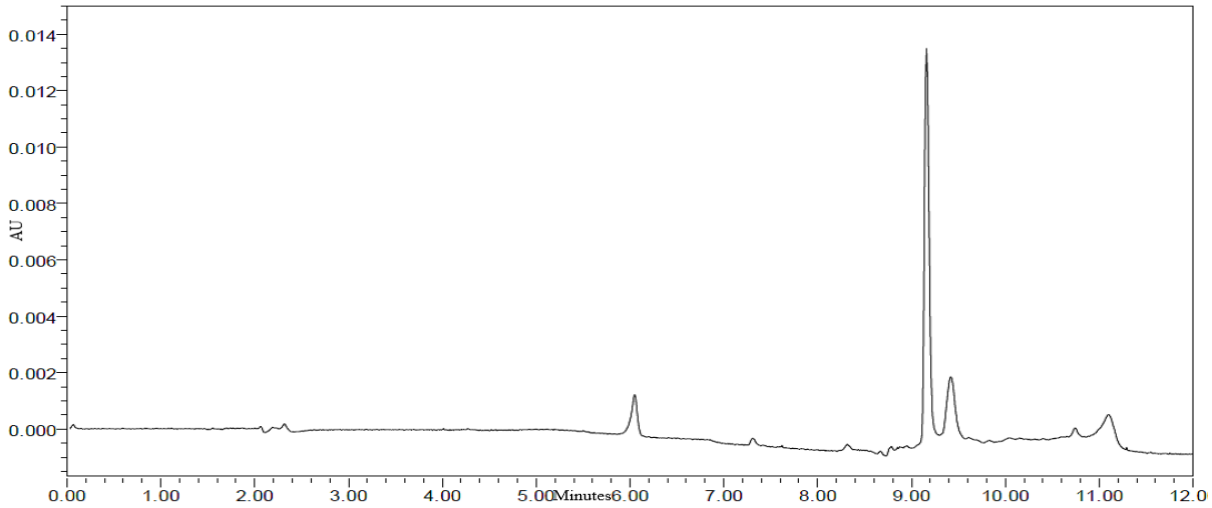

Figure 9: Chromatogram of LOQ solution of Lamotrigine

ROBUSTNESS

Robustness for analytical method was performed by changing the column temperature ±5°C from normal temperature (30 °C) and flow rate from normal flow rate (1ml/min) of mobile phase.

Robustness Data Table						
S.No.	**Temp**	**Flow**	**R.T. (min)**	**Area**	**K Prime**	**USP Plate Count**
1	25^0C	1.0ml/min	6.447	1243879	5.447	61678
2		1.0 ml/min	6.437	1238983	5.437	61175
3		1.0 ml/min	6.444	1245043	5.444	60702

4	30^0C	1.0ml/min	6.361	1241951	5.361	59051
5		1.0 ml/min	6.398	1228800	5.398	60433
6		1.0ml/min	6.422	1237536	5.422	62077
7	35^0C	1.0ml/min	6.307	1223074	5.307	62390
8		1.0 ml/min	6.263	1242989	5.263	52462
9		1.0ml/min	6.346	1232431	5.346	59803
10	30^0C	0.9ml/min	7.008	1393056	6.007	72592
11		0.9ml/min	6.994	1403204	5.99	69771
12		0.9ml/min	7.006	1390723	6.00	69503
13	35^0C	1.1ml/min	5.928	1143468	4.927	42399
14		1.1ml/min	6.003	1137091	5.00	48568
15		1.1ml/min	6.036	1143452	5.036	51535

Table 7: Robustness Data

RUGGEDNESS

System-to-system, analyst-to-analyst, and column-to-column variability studies were carried out for the method's ruggedness under comparable circumstances at various points in time. Over the course of two days, a research was done to determine the stability of standard and test preparation on a bench top. It verifies that during the course of the two-day experiment, the standard and test solution remained stable (Sanjay et al.,2013). When two distinct HPLC systems, columns, and analysts are compared, it becomes clear that the associated test technique is robust against variability in each of these variables from system to system, analyst to analyst, and column to column.

Forced Degradation Studies

The degradation study of Lamotrigine is carried out in the solvent system, acidic conditions and in oxidative conditions at room temperature and elevated temperature 80^0C. For this 1% solution of acid & peroxide was prepared and 1ml of working standard was taken. Equivalent quantities of durg and 1% solutions of acid & peroxide were mixed in three different vials for forced degradation studies (Selvadurai et al.,2012).

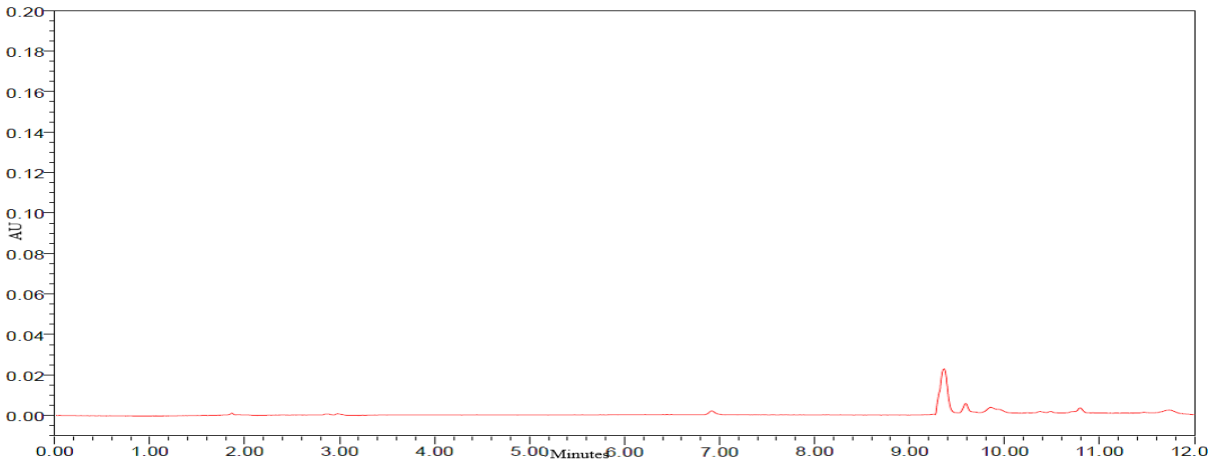

Figure 10: Blank 1N HCL Chromatogram

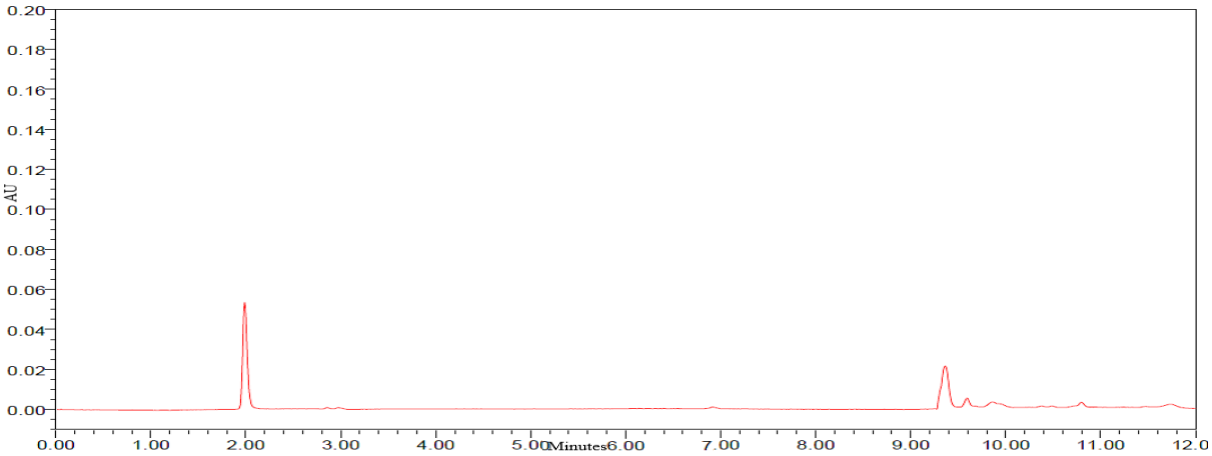

Figure 11: Blank H_2O_2 Chromatogram

Day 1 study of drug under acid, peroxide condition & at 80^0c temp

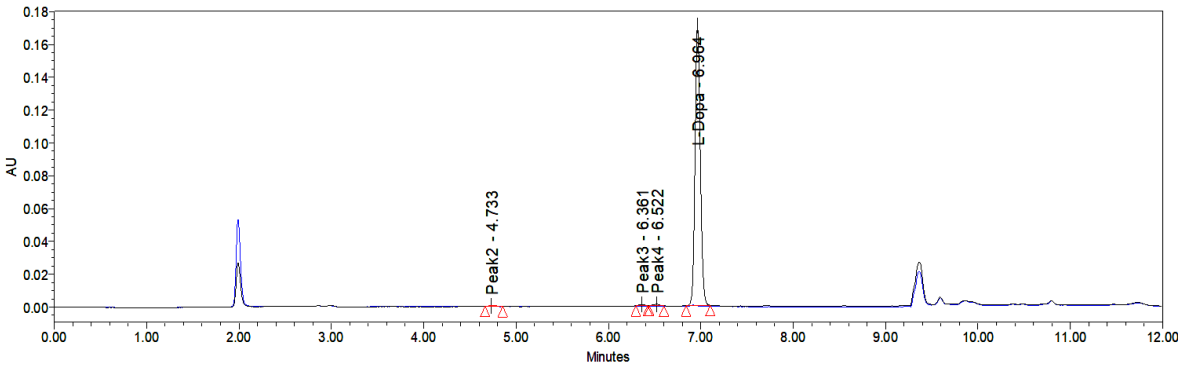

Figure 12: Chromatogram of 1 Day of drug with H_2O_2

No.	Name	Retention Time	Area	% Area	Height
1	Peak2	4.733	2752	0.40	424
2	Peak3	6.361	3338	0.49	804
3	Peak4	6.522	4614	0.67	910
4	Lamotrigine	6.964	676332	98.44	170623

Table 8: Chromatographic result at end of 1 Day of drug with H_2O_2

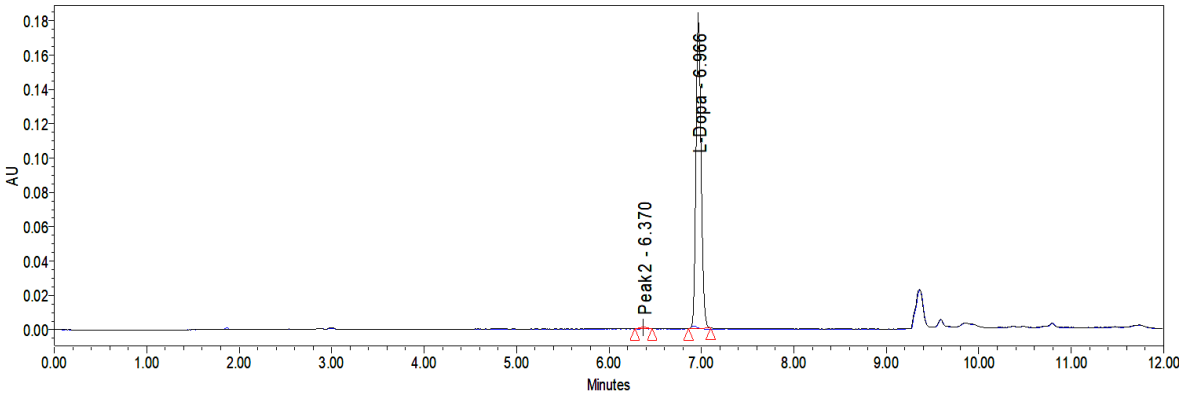

Figure13: Chromatographic result at the end of day $1^{st}$with HCl

	Name	Retention Time	Area	% Area	Height
1	Peak2	6.370	5224	0.74	1037
2	Lamotrigine	6.966	698547	99.26	179225

Table 9: Chromatographic result at the end of day 1st with HCl

Analysis of Quality Control Solutions

Three injections of each concentration were used to create quality control solutions for lamotrigine in the conc. of low (250 ppm), medium (350 ppm), and high (450 ppm). By contrasting the data from standard solutions the quality control solutions was formed. It was possible to determine if the instrument produced consistent findings or not and to assess the instrument's performance prior to the analysis of test solutions (Youssef etal.,2007).

Results and discussion:

Method Validation

In accordance with globally recognized ICH principles, the designed and improved HPLC technique was validated with regard to characteristics including linearity, specificity, accuracy, precision, and stability, limit of detection (LOD) and limit of quantification (LOQ).

Specificity

The comparison between the chromatograms of Lamotrigine standard stock solution sample and chromatogram of Lamotrigine test samples and the blank solution determines the specificity of the method. No additional peak showed at the drug's Rt, indicating that there was no interference from contaminants, according to the specificity investigation.

Linearity

Different medication concentrations were created for linearity experiments. Peak area was used to measure the reaction. Replicate analysis at five concentration levels produced the calibration plot, and the Microsoft Excel® program's least squares approach was used to compute the linear regression equation. Plotting the graph of the peak area (y) against the concentration of analyte (x) in µg/ml yielded a calibration curve that was found to be linear in the concentration range of 50 to 250ppm. The equation for linear regression is:

$y = ax \pm b$

Where “a” represents the curve's slope and “b” denotes its intercept. Based on the following results, the usual linear regression is determined as:

$Y = 6E + 06x + 91344$

The value of correlation coefficient for the drug is discovered to be 0.9901which is acceptable.

Accuracy

The study of percentage recovery is used to assess the method's accuracy. Recovery is usually ascertained by contrasting the method's reaction with known amount given to the substance to a reference substance. A low recovery of the assay, which is less than 90%, would be a strong sign that the procedure is problematic. The developed procedure for Lamotrigine is a legitimate approach.

Precision

The precision of this procedure was analysed by taking results of standard deviation or relative standard deviation of anlyte based on 6 injections of standard solution and 2 different concentration of drug. If the assay's percentage RSD is greater than 2% then the developed procedure is not reliable or vice versa.

Stability

Analyte solutions were treated under various circumstances (alkali, acid, peroxide, and increased temperature) at intervals of the first, second, third, and fourth day in order to ascertain the stability of the analyte solution.

Compare the peak areas of the analyzed chromatograms from Days 1 and 4 with the peak areas of fr esh solutions from Day 1 to determine the stability of the solutions.Following four days of testing in various environments; acid peroxide, alkali and high temperature, It was found out that Lamotrigine had been destroyed in both acid and peroxide conditions as well as in high temperature (80^0C) over the full 4 days of the trial.

Limit of detection (LOD) and Limit of qualification (LOQ)

Drugs	LOD(ppm)	LOQ(ppm)
Lamotrigine	5	7.7

Table 10: LOD calculation

Robustness

Robustness is when the same sample is analyzed under a range of standard test conditions, such as different analysts, laboratories, instruments, reagents, assay temperatures, minor variations in the mobile phase, different days etc.Retention time got decreased and the area got increased to an acceptable limit of 0.8 10 1.2ml after adjusting the temperature and flow rate, as flow rate increased from 0.9 to 1.1 ml/min.

Conclusion

Standard drug sample of Lamotrigine was identified and characterized by various spectroscopic methods and they were found to be highly pure. Standard sample of drug was utilized as reference standard. Validation of the developed and optimized HPLC method was carried out in the light of ICH Guidelines with respect to parameters- linearity, specificity, accuracy, precision, robustness, limit of detection (LoD) and limit of quantification (LoQ).

The proposed method was stable during analysis. The stability of the analyte solution was determined at interval of 1st, 2nd, 3nd, and 4th day. The low values of %recovery and %C.V. showed that the method is precise within the acceptance limit of 5% (according to ICH guidelines). Developed assay method is simple, rapid, accurate, precise, economical, specific and reproducible for the qualitative and quantitative determination of Lamotrigine with good resolution in short time and high sensitivity.It was concluded that the developed method offers several advantages such as rapidity, simple mobile phase and sample preparation step, improved sensitivity makes it specific and reliable for its intended use. This method can be applied to analysis of pharmaceutical dosage forms.

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