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Development and Validation of New Analytical Methods for the Simultaneous Estimation of Selected Combinations of Drugs in Pharmaceutical Formulations

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Abstract: *This paper proposes a high-performance liquid chromatography (HPLC) method for the simultaneous estimation of nortriptyline and gabapentin. The analytical conditions of the method were selected based on the chemical nature of the two compounds, and the method was validated through various studies including system suitability, specificity, linearity, LOD/LOQ, and recovery studies. The proposed method uses a Zorbax C18 column, a phosphate buffer with an acidic pH, and acetonitrile as the organic constituent of the mobile phase. The system suitability test evaluated the suitability of the chromatographic system for the analysis of the drug combination, and the specificity of the method was evaluated by assessing interference from excipients in the pharmaceutical dosage form prepared as a placebo solution. The linearity of the method was evaluated through linear regression analysis of the standard curve, and the LOD and LOQ values were determined by serial dilutions of nortriptyline and gabapentin stock solutions. The recovery studies were conducted to evaluate the degree of accuracy of the proposed method through known amounts of standard nortriptyline and gabapentin added to preanalyzed samples. Overall, the proposed HPLC method offers a reliable and accurate means for the simultaneous estimation of nortriptyline and gabapentin.*

Keywords: HPLC method, Nortriptyline, Gabapentin, Validation, System suitability, Specificity

I. INTRODUCTION

The development and validation of new analytical methods for the simultaneous estimation of selected combinations of drugs in pharmaceutical formulations is a critical area of research in the pharmaceutical industry. The accurate and precise quantification of multiple drug components in a single formulation is essential for ensuring the safety and efficacy of the final product.[1-5]

The simultaneous estimation of multiple drugs in pharmaceutical formulations is a complex task due to the presence of several drug components, excipients, and potential interferences from the sample matrix. Traditional analytical methods often involve the separation and quantification of individual drug components, which can be time-consuming and may not accurately reflect the true composition of the formulation. As a result, there is a growing need for analytical methods that can simultaneously estimate multiple drugs in pharmaceutical formulations.[5-13]

The development of new analytical methods for the simultaneous estimation of selected combinations of drugs requires a multidisciplinary approach that combines analytical chemistry, pharmacology, and statistics. Researchers must carefully select appropriate analytical techniques, sample preparation methods, and analytical standards to ensure accurate and precise quantification of the drug components.[14,15]

One of the primary challenges in developing new analytical methods is the potential for interference from other components in the sample matrix. These interferences can lead to inaccurate or imprecise measurements of drug concentrations, which can in turn affect the safety and efficacy of the final product. Researchers must carefully select appropriate sample preparation techniques and chromatographic conditions that can minimize the potential for interferences.[16,17]

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In addition to the technical challenges of developing new analytical methods, there are also regulatory considerations that must be taken into account. Pharmaceutical companies must follow specific regulations and guidelines for the development and validation of analytical methods. These regulations and guidelines are designed to ensure the safety and efficacy of pharmaceutical products and to promote consistency and standardization across the industry.

The validation of analytical methods is a critical step in the process of developing new analytical methods for the simultaneous estimation of selected combinations of drugs. The validation process involves a series of tests and experiments designed to assess the accuracy, precision, and reliability of the analytical method. These tests typically include assessments of linearity, specificity, accuracy, precision, and robustness, among other parameters.[18-25]

The validation process also involves the establishment of appropriate acceptance criteria for each of these parameters. These acceptance criteria are based on the specific requirements of the analytical method and the intended use of the final product. For example, an analytical method for the simultaneous estimation of multiple drugs in a pharmaceutical formulation intended for human use would likely have more stringent acceptance criteria than an analytical method for the same drugs in an animal feed supplement.[26-28]

The development and validation of new analytical methods for the simultaneous estimation of selected combinations of drugs in pharmaceutical formulations has important implications for the pharmaceutical industry. Accurate and precise quantification of multiple drug components in a single formulation can help to reduce the risk of adverse effects and improve the efficacy of the final product. In addition, the development of new analytical methods can help to improve the efficiency and speed of drug development and manufacturing processes.

In conclusion, the development and validation of new analytical methods for the simultaneous estimation of selected combinations of drugs in pharmaceutical formulations is an important area of research in the pharmaceutical industry. By carefully selecting appropriate analytical techniques, sample preparation methods, and analytical standards, researchers can develop accurate and precise analytical methods that meet the specific requirements of the pharmaceutical industry. The validation of these methods is a critical step in the process and involves a series of tests and experiments designed to assess the accuracy, precision, and reliability of the analytical method. The development of new analytical methods has important implications for the safety, efficacy, and efficiency of drug development and manufacturing processes[29,30]

II. MATERIALS AND METHODS

2.1 Mobile Phase Preparation

Accurately weighed and transferred 2.72g of potassium dihydrogen ortho phosphate and 0.525g of dipotassium hydrogen phosphate to a 1000 ml volumetric flask, 300 ml water was added and the volume was made up to 1000 ml with water. The pH was adjusted to 4.2. Buffer and acetonitrile were mixed in the ratio of 70:30% v/v. The solution was filtered through a 0.45µm membrane filter and degassed for further use.

2.2 Standard Preparation

Standard stock solution containing nortriptyline (100mcg/ml) and gabapentin (1000mcg/ml) was prepared by transferring accurately weighed 10mg of nortriptyline and 100mg gabapentin working standard powder into a 100ml volumetric flask. 40ml of diluent was added to the flask, sonicated and cooled to room temperature. This solution was diluted to the mark with same diluent. Working standard solutions containing 60-180µg/mL of nortriptyline and 300-900µg/mL of gabapentin were prepared by pipetting aliquots of this stock solution into a 10ml volumetric flask and diluted up to the mark with the same diluent.

2.3 Assay of Formulation

Twenty tablets of Gabapin NT (Label claim;Gabapentin-100mg and nortriptyline-10mg) were accurately weighed & powdered. The quantity equivalent to 10mg of nortriptyline and 100mg of Gabapentin were transferred to 100ml amber colored volumetric flask and to this 60ml of distilled water was added & sonicated for 15 min at room temperature & then diluted to the mark with distilled water. The sample solution was filtered through whatmann filter paper prior to use. Each of the solutions (20µL) was injected six times into the column. From the peak areas, the drug content in tablets were quantified using the regression equation obtained from pure sample.

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Validation According to ICH guideline Linearity

For quantitative analysis of Drug, the calibration curves were plotted for each concentration ranges. The linearity ranges for Drug found to be 50-150 µg/ml respectively.

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

The LOD and LOQ of Drug by the proposed methods were determined using calibration standards. LOD and LOQ values were calculated as 3.3 SD/D and 10 SD/D respectively, where D is the slope of the calibration curve and SD is the standard deviation.

Accuracy

The accuracy was determined by standard addition method. Three different levels (80%, 100% and 120%) of standards were spiked to Capsules formulation in triplicate. The mean of percentage recoveries and the %RSD was calculated.

Precision

The reproducibility of proposed method was determined by performing Capsule assay at different time intervals (3 hour interval) on same day (Intra-day precision) and onthree different days (Inter-day precision) Drug.

Robustness and Ruggedness

The Robustness study was carried out by determining effect of small variation in wavelengths and in Ruggedness; sample was analysed by two different analysts.

III. RESEULT AND DISCUSIONS

DEVELOPMENT AND OPTIMIZATION OF THE HPLC METHOD:

The analytical conditions for the proposed method were selected, basing on the chemical nature of nortriptyline and gabapentin.

Initial spectroscopic analysis of compounds showed that nortriptyline and gabapentin showed a maximum UV absorbance (λmax) at 221nm and 231nm respectively. Therefore, the chromatographic detection was performed at 231nm using a photo diode array detector as both the compounds showed good response at this wavelength. The development trials of each component were carried, by keeping them in various extreme conditions.

The column selection has been done on the basis of back pressure, resolution, peak shape, theoretical plate, day-to-day reproducibility of the retention time and the resolution between nortriptyline and gabapentin peak. After evaluating all these factors, Zorbax C18 column(250 mmx4.6 mm I.D; particle size 5µm) was found to be suitable as it gave satisfactory results. The selection of buffer is based on chemical nature of both the drugs. The acidic pH range was found to be suitable for solubility, resolution, stability, theoretical plates and peak shape of both components. Best results were obtained with phosphate buffer (4.2) for nortriptyline and gabapentin. Acetonitrile was chosen as organic constituent of mobile phase, to reduce the longer retention time and to attain good peak shape. Preliminary trials using different composition of mobile phases consisting of buffer (4.2) and acetonitrile in the ratio of 60:40 v/v and 50: 50 v/v, that did not give good peak shape for nortriptyline and gabapentin.

Finally, the best separation and resolution of nortriptyline and gabapentin is achieved by fixing the mobile phase composition consisting of a mixture of buffer and acetonitrile in the ratio of 70:30 v/v . Under these conditions nortriptyline and gabapentin were eluted at 2.882 and 3.629 minutes respectively with a run time of 4 min. Optimized mobile phase proportion provided good resolution between nortriptyline and gabapentin and the flow rate was maintained at 1.0ml/min and the eluents were monitored at 231nm. The typical HPLC chromatogram for simultaneous estimation of nortriptyline and gabapentin standard by using the aforementioned chromatographic conditions is represented in Fig 6.1 System suitability results of the method are presented in Table.6.1.

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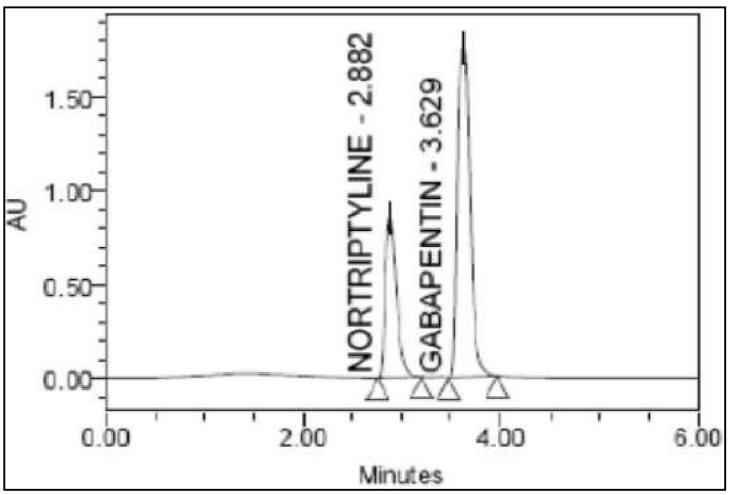

Fig: 1. Typical HPLC Chromatogram Showing The Peaks Of Nortriptyline And Gabapentin

METHOD VALIDATION SYSTEM SUITABILITY:

A system suitability test of the chromatographic system was performed before each validation run. Five replicate injections of standard preparation were injected and the column efficiency, resolution and peak asymmetry were calculated for the standard solutions (Table:5.01). The values obtained demonstrated the suitability of the system for the analysis of this drug combinations, system suitability parameters may fall within ± 3.0 % standard deviation range and % RSD less than 2.0 during routine performance of the method.

Table 1: system suitability parameters

Parameters	**Nortriptyline**	**Gabapentin**
Retention time	2.882	3.629
USP plate count	3611	3694
USP Tailing	1.432	1.5 42
Linearity Range(µg/ml)	60-180	300-900
Limit of detection (LOD) (µg/ml)	0.0013	0.007
Limit of quantitation (LOQ) (µg/ml)	0.004	0.024

SPECIFICITY:

The specificity of the method was evaluated by assessing interference from excipients in the pharmaceutical dosage form prepared as a placebo solution. The peak purity of the nortriptyline and gabapentin (Fig:5.04.b) were found satisfactory under different stress conditions as there was no interference of any peak of degradation product with drug peak.

LINEARITY OF DETECTOR RESPONSE:

The standard curve was obtained in the concentration range of 60-180µg/ml for nortriptyline and 300-900µg/mL for gabapentin. Chromatograms obtained during linearity study were shown in [Fig:5.05]. The linearity of this method was evaluated by linear regression analysis. Slope, intercept and correlation coefficient [r2] of standard curve were plotted and calculated and are given in Fig:5.06.a & Table:5.02 for nortriptyline and Fig:5.06.b & Table:5.02 for gabapentin demonstrating the linearity of the proposed method.

LOD & LOQ:

The limit of detection and limit of quantification were evaluated by serial dilutions of nortriptyline and gabapentin stock solution in order to obtain signal to noise ratio of 3:1 for LOD and 10:1 for LOQ. The LOD values for nortriptyline and gabapentin were found to be 0.0013µg/mL and 0.007µg/mL, respectively and the LOQ values

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0.004µg/mL and 0.024µg/mL and are reported in Table:5.02 respectively. The chromatograms of LOD and LOQ study were shown in Fig:5.07.a&b.

PRECISION:

Data obtained from precision experiments are given in Table.5.03 for intraday and interday precision study for both nortriptyline and gabapentin. The RSD values for intra-day precision study and inter-day precision study was < 2.0 % for nortriptyline and gabapentin confirming that the developed RP-HPLC method was precise. The results of precision studies are summarized in Table.5.03

ACCURACY:

Known amounts of standard nortriptyline and gabapentin added to preanalyzed samples and were subjected to the proposed HPLC method at 50%, 100% and 150% to evaluate the degree of accuracy. The Results of recovery studies are reported in Fig:5.09.a-c & Table.5.04.a&b. From the data reported in Table:5.04.a&b the mean recovery data obtained for each level as well as for all levels combined were within 2.0% of the label claim for the active substance with an %RSD < 2.0%, which satisfied the acceptance criteria set for the study

ROBUSTNESS:

To evaluate the robustness of the developed RP-HPLC method, small deliberate Variations in the optimized method parameters were done. The effect of change in flow rate and mobile phase ratio on the retention time and tailing factor were studied. The results of this robustness study were reported in Table: 5.05. The values for proposed method are well within acceptance limits of 98-102%, with a RSD of less than 2.0%. It was also observed that there were no marked changes in chromatograms, which demonstrated that the developed method was robust in nature.

ASSAY OF FORMULATION:

Twenty tablets were accurately weighed & powdered. The quantity equivalent to Gabapentin-100mg + nortriptyline - 10mg were transferred to 100ml amber colored volumetric flask and to this 60ml of distilled water was added & sonicated for 15 min at room temperature and then diluted up to the mark with distilled water. The sample solution was filtered through whatmann filter paper prior to use. Each of the solutions (20µL) were then injected five times into the column. From the peak areas, the drug content in tablets were quantified using the regression equation obtained from pure sample and the relevant results are shown in Table:5.06 respectively.

Fig 2`: CHROMATOGRAM OF BLANK OF NORTRIPTYLINE AND GABAPENTIN

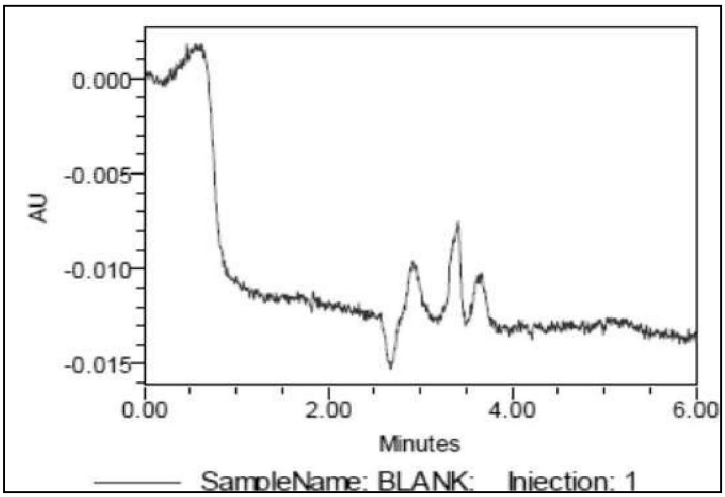

Fig 3: CHROMATOGRAM OF PLACEBO OF NORTRIPTYLINE AND GABAPENTIN

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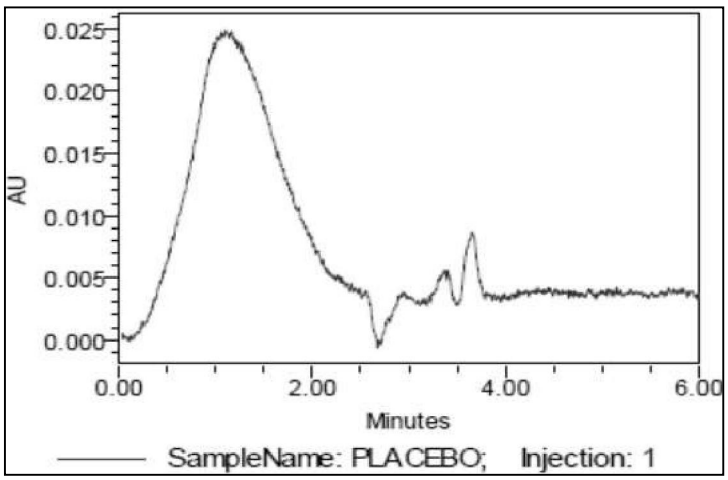

Fig 4: LINEARITY CHROMATOGRAM OF NORTRIPTYLINE AND GABAPENTIN

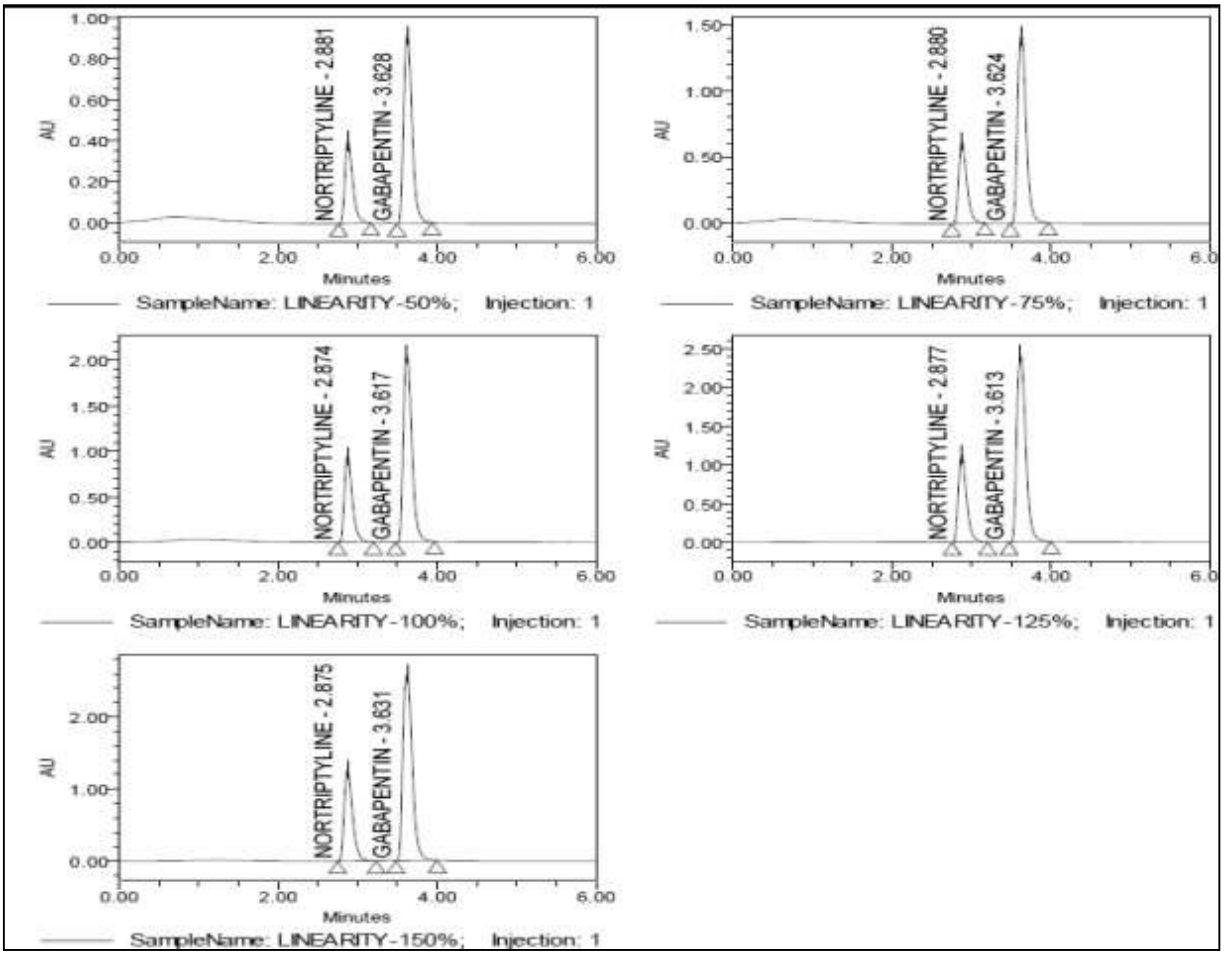

Fig 5: LINEARITY CHROMATOGRAM OF NORTRIPTYLINE

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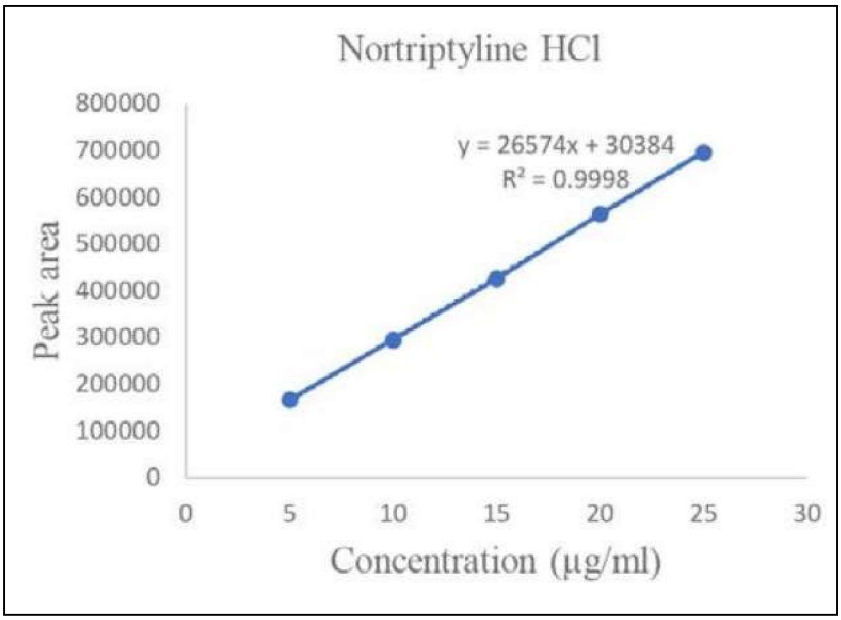

Fig 6: LINEARITY CHROMATOGRAM OF GABAPENTIN

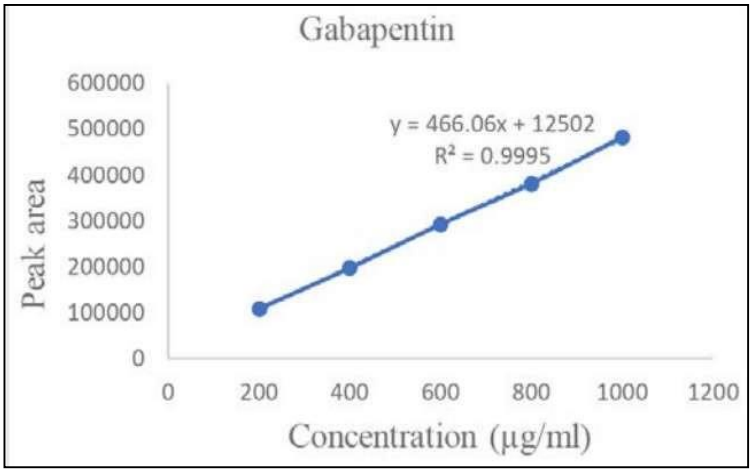

Fig 7: CHROMATOGRAM OF LOD STUDY OF NORTRIPTYLINE AND GABAPENTIN

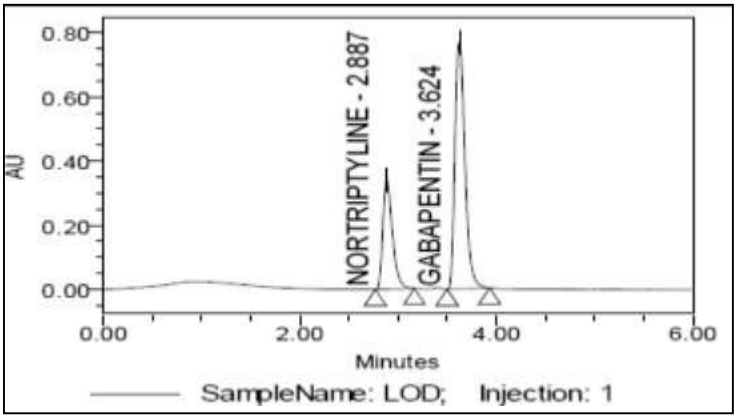

Fig 8: CHROMATOGRAM OF LOQ STUDY OF NORTRIPTYLINE AND GABAPENTIN

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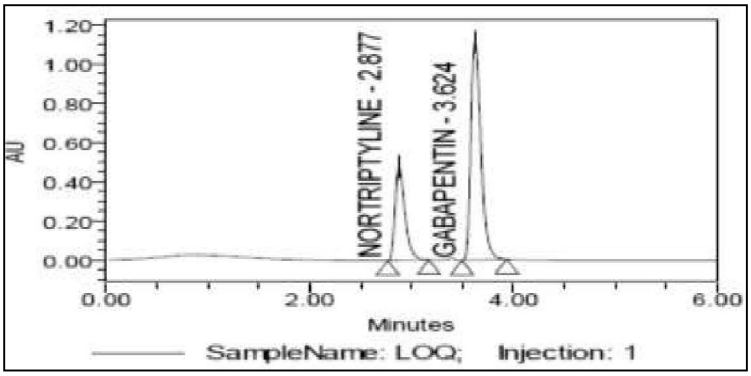

Fig 9: PRECISION STUDY CHROMATOGRAMS OF NORTRIPTYLINE AND GABAPENTIN (REPLICATE:1-6)

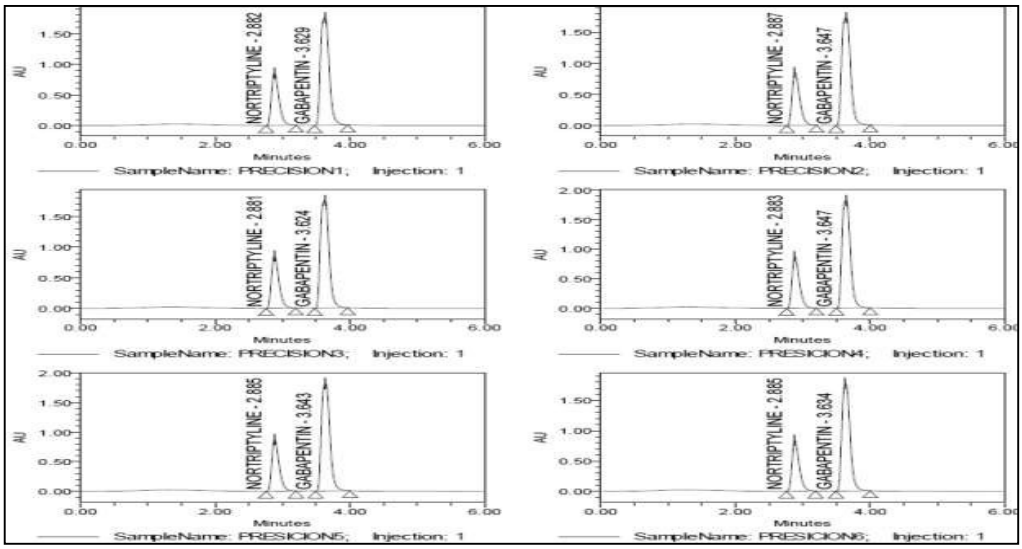

Fig 10: ACCURACY STUDY CHROMATOGRAMS OF NORTRIPTYLINE AND GABAPENTIN AT LEVEL-1 (50%)

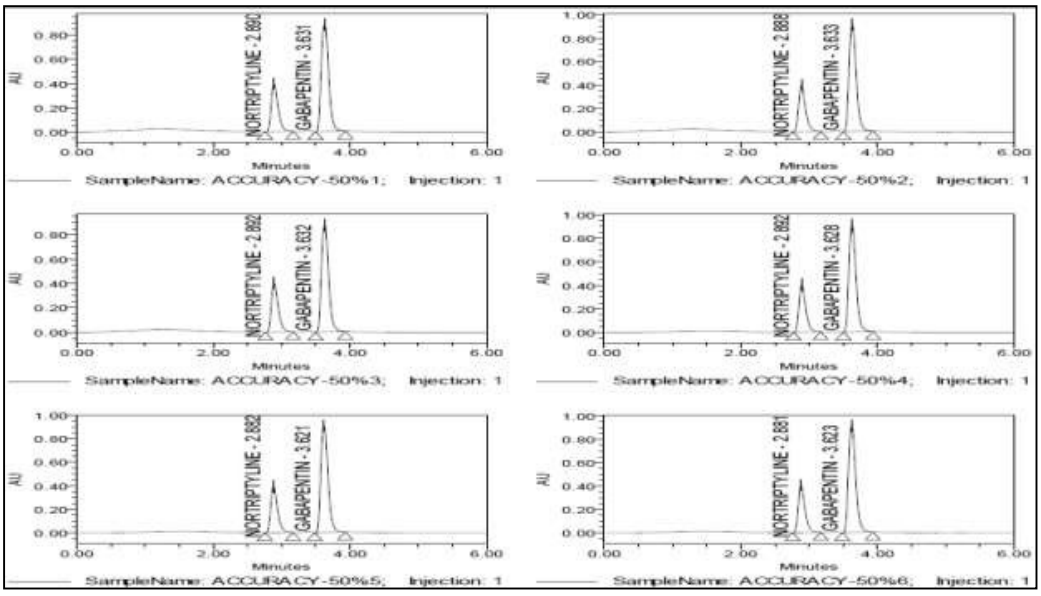

Fig 11: ACCURACY STUDY CHROMATOGRAMS OF NORTRIPTYLINE AND GABAPENTIN AT LEVEL-2 (100%)

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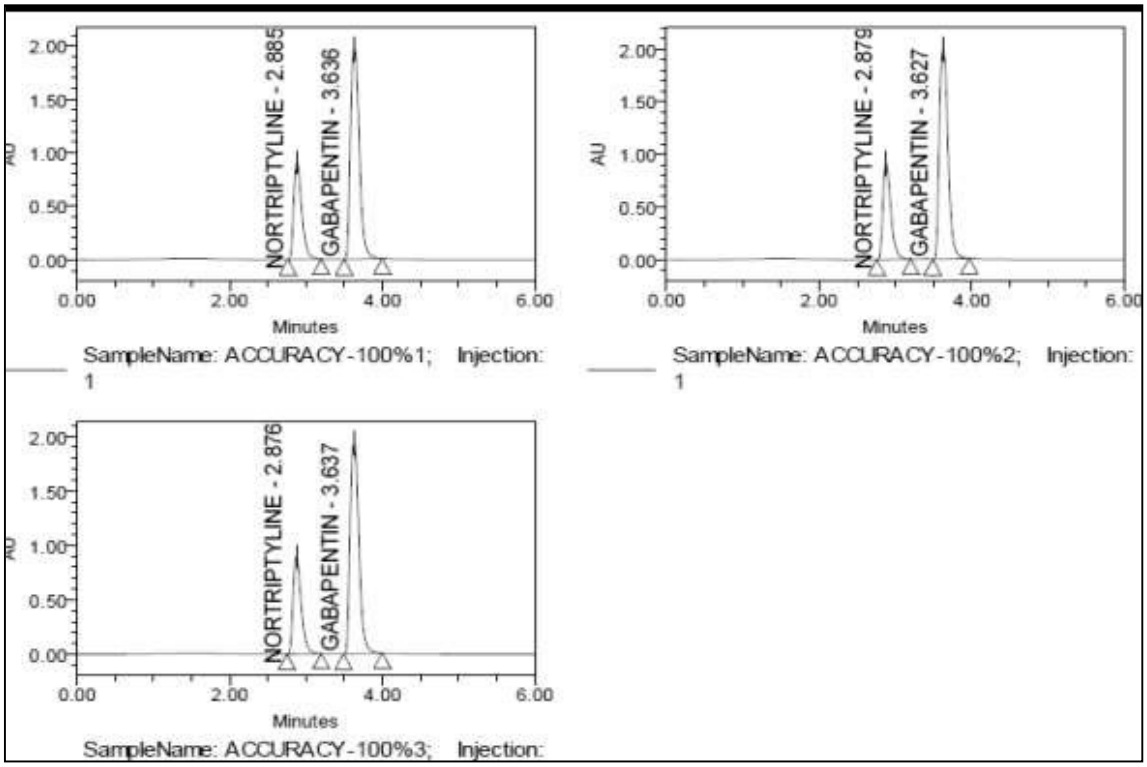

Fig 12: ACCURACY STUDY CHROMATOGRAMS OF NORTRIPTYLINE AND GABAPENTIN AT LEVEL-3 (150%)

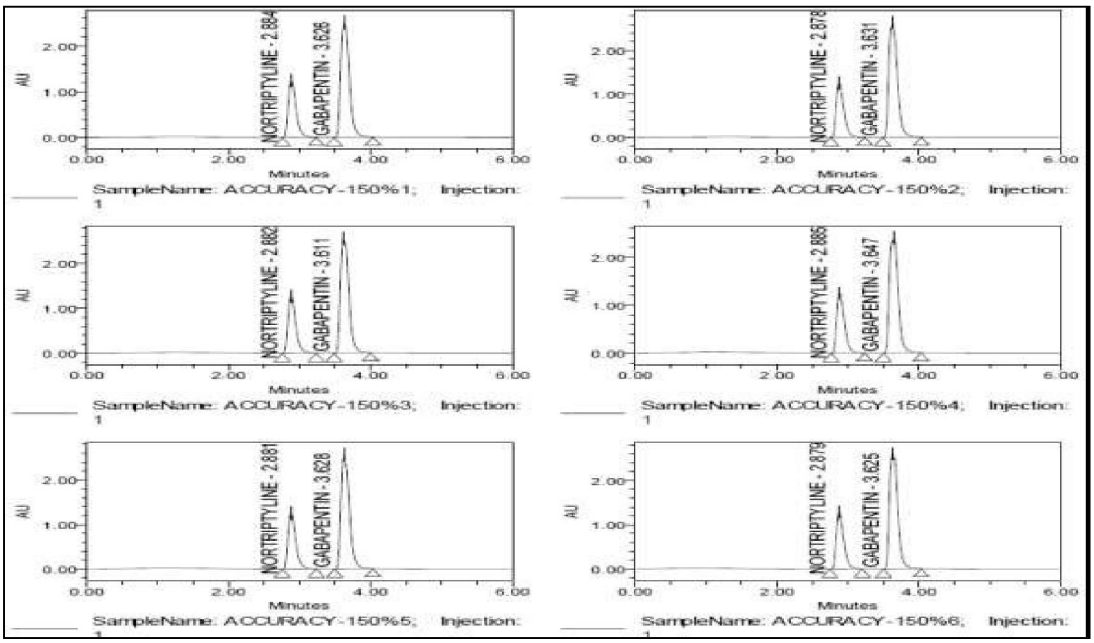

Fig 12: ROBUSTNESS STUDY CHROMATOGRAMS OF NORTRIPTYLINE AND GABAPENTIN AT FLOW RATE AND TEMPERATUR

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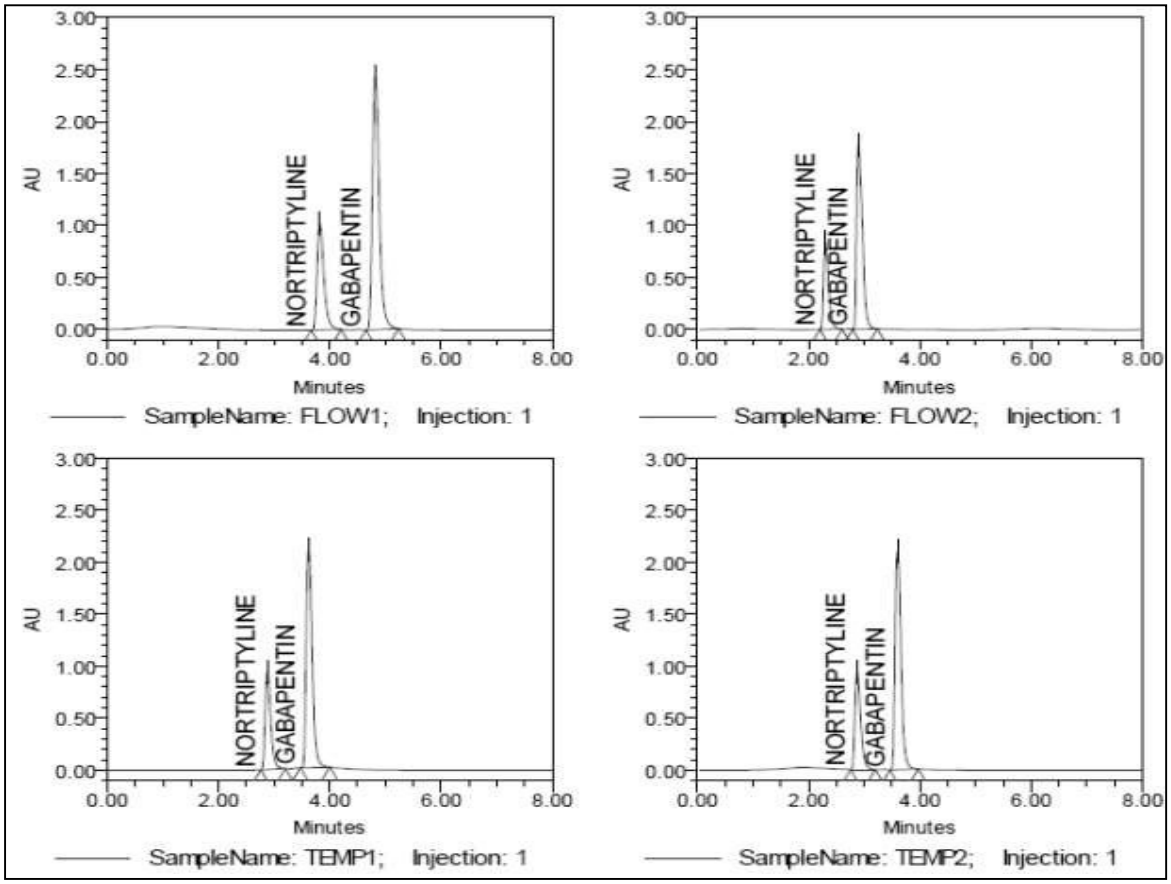

TABLE.2: SYSTEM SUITABILITY PARAMETERS

Parameters	Nortriptyline	Gabapentin
Retention time	2.882	3.629
USP plate count	3611	3694
USP Tailing	1.432	1.5 42
Linearity Range(µg/ml)	60-180	300-900
Limit of detection (LOD) (µg/ml)	0.0013	0.007
Limit of quantitation (LOQ) (µg/ml)	0.004	0.024

TABLE 3 : LINEARITY STUDIES OF NORTRIPTYLINE AND GABAPENTIN BY THE PROPOSED METHOD

LINEARITY STUDY FOR NORNTRIPTYLINE			**LINEARITY STUDY FOR GABAPENTINE**		
%Level (approx.)	**Conc. (µg/ml)**	**Area**	**%Level (approx.)**	**Conc. (µg/ml)**	**Area**
50	60		50	300	7894616
75	90		75	450	11826470
100	120		100	600	15705853
125	150		125	750	19777554
150	180		150	900	23658461

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Slope	54787	**Slope**	263191
Intercept	2851.2	**Intercept**	18919
RSQ(r^2)	1.0000	**RSQ(r^2)**	0.9999
LOD	0.0013	**LOD**	0.007
LOQ	0.004	**LOQ**	0.024

TABLE 4: PRECISION DATA OF NORTRIPTYLINE AND GABAPENTIN

	NORNTRIPTYLINE	**GABAPENTINE**
	Peak area	**Peak area**
PRECISION	6578066	15749140
	6574421	15765459
	6579352	15779528
	6574187	15791995
	6576920	15778096
	6573077	15766303
Average*	6576004	15768420
SD*	2476.882	18124.24
%RSD*	0374	0.110

* Average of six determinations

TABLE.5 : RECOVERY STUDIES OF THE PROPOSED RP-HPLC METHOD.

Concentration of nortriptyline (µg/ml)	**Amount added (µg/ml)**	**Total amount (µg/ml)**	**Amount found* (µg/ml)**	**% Recover***	**Mean***
120	20	140	139.89	99.92%	99.94%
120	40	160	159.99	99.93%	
120	60	180	179.95	99.97%	

* Average of three determinations

TABLE.6 b:RECOVERY STUDIES OF THE PROPOSED RP-HPLC METHOD

Concentration of Nortriptyline (µg/ml)	**Amount added (µg/ml)**	**Total amount (µg/ml)**	**Amount found* (µg/ml)**	**% Recover***	**Mean***
900	**100**	**1000**	**999.89**	**99.98%**	**99.95%**
900	**200**	**1100**	**1098.99**	**99.90%**	
900	**300**	**1200**	**1199.98**	**99.99%**	

Average of three determinations

TABLE 7 : RECOVERY STUDIES OF THE PROPOSED RP-HPLC METHOD

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Robust condition		NORNTRIPTYLINE			GABAPENTINE		
Flow rate		Theoretical plate	RT	Peak Area	Theoretical plate	RT	Peak Area
	0.8 ml/min	2744	2.888	6566165	4030	1.688	15797961
	1.2 ml/min	2912	2.882	6593931	4095	1.567	15804738
Temp	40^0 C	2938	2.880	6574779	4070	1.573	15864008
	45^0 C	2795	2.799	6575245	4148	1.603	15767369

TABLE 8 : ANALYSIS OF MARKETED TABLETS BY THE PROPOSED METHOD

DRUG	LABEL CLAIM	QUANTITY FOUND*	%RSD	%ASSAY
NORNTRIPTYLINE	100	99.222	0.0237	99.22
GABAPENTINE	10	99.822	0.0849	99.82

IV. CONCLUSION

In conclusion, the proposed HPLC method provides an efficient and accurate means for the simultaneous estimation of nortriptyline and gabapentin in pharmaceutical formulations. The validation studies confirmed the reliability and specificity of the proposed method, which makes it suitable for routine analysis in quality control laboratories. The proposed method can also be applied to other matrices, such as biological samples, for pharmacokinetic studies. Further research can focus on the application of the proposed method for the analysis of other drug combinations and the development of alternative methods that can reduce analysis time and improve sensitivity.

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