NEWER RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF LAFUTIDINE AND RABEPRAZOLE IN DOSAGE FORM

Dr.Dillip Kumar Jena[1]*, Lelin Behra[2] , Mrs. Lizalin Behera[3]

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Abstract

For the validation of Lafutidine and Rabeprazole, each in their unique structure and as tablets, a speedy and fruitful contrary stage elite execution fluid chromatographic method has been communicated. For the chromatography, methanol used to be mixed with a Phenomenex Gemini C18 (4.6 x 250 mm) 5 fragment. The ID was once carried out at 230 nm with a floating fee of 1.0 ml/min with the use of a TEA Pad (65:35 v/v) as the sensible stage. Lafutidine and Rabeprazole had a quick resource season of 2.121, and 3.643 0.02min, respectively. For Lafutidine concentrations between 10 and 50 mg/ml and Rabeprazole concentrations between 20 and one hundred mg/ml, the technique yields linear responses. The method's RSD for the size declaration used to be 2% or less. This shape is useful for making sure of constant mass and remedy definitions.

Words to remember: rabeprazole, lafutidine, reverse segment excessive overall performance liquid chromatography, and recommendation.

[1*]Department Of Pharmaceutical Chemistry Principal, Luckky College Of Pharmaceutical Sciences, Bhubaneswar
[2]Associate Professor, Indira Gandhi Institute of Pharmaceutical Sciences, Bhubaneswar
[3]Assistant Professor, Koustuv Research Institute of Medical Science (KRIMS)

***Corresponding Author:** Dr.Dillip Kumar Jena[1]
*Department Of Pharmaceutical Chemistry Principal, Luckky College Of Pharmaceutical Sciences, Bhubaneswar.

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INTRODUCTION

Evaluation is a catch-all period for the talent and lookup worried in settling on the format of materials in phrases of its components or blends. Science is now not primarily based on empirical proof but on learning about artificially produced proof and affirmation of the relationship (atomic, sub-nuclear) of substances, materials, and their compound plan. All ordinary activities and cycles, which are the clarification for life, are constructed from manufactured combinations and metal particles. Peptides, proteins, carbohydrates, lipids, and nucleic acids are all observed in all components of the body, whereas some of the different naturally taking place combinations and particles (endogenous species) are solely discovered in hint quantities in one place of the body. For proper lookup to succeed, it ought to center attention on one thing: refining techniques that have already been permitted in precept and that permit for a specific emotional and quantitative appraisal of materials. Science, authentic science, microbiological science, atomic science, and technological know-how all make contributions to the benchmarks used through clever science. This technique offers records on the absolute estimation of one of these components. Nation states alter and standardize countrywide fitness care and pharmaceutical help programs. These recommendations are posted in books referred to as "Pharmacopoeia" (for instance, the International Pharmacopoeia (IP), the United States Pharmacopoeia (USP), and the British Pharmacopoeia (BP)). Quantitative compound evaluation is a vital device for making positive that the marketed characteristics of normally offered and used herbal resources are met. Many distinct prescriptions are well-versed in the marketplace. Although the significance of exceptional in all merchandise and redress is undeniable, it is of paramount significance in prescribed drugs due to their direct impact on human life. The inclusion of drug thinking in pharmacopeias takes place after its introduction to the market. Reasonable shortfalls in the anticipated and extra big utilization of these

remedies, reviews of new hazardous substance levels, and development in affected person monitoring and show of most efficient medicines through rivals all make contributions to this situation. It's viable that there are no tried-and-true strategies for administering these drugs as prescribed via pharmacopeias. To decide a substance's compound formation, instrumental evaluation takes its bodily houses into account. Drug checking out requires maintaining thinking about the methods vital to decide the identity, potency, quality, and excellence of compounds with therapeutic value. two Prescriptions (drugs and their definitions) are at the coronary heart of remedy evaluation, however so are their predecessors, such as the herbal substance whose presence or absence determines a medicine's efficacy and character. The idea was once no longer resolved even after laying out its credibility through uprightness checking out and deciding on the notion of the unadulterated ingredient in the remedy and its arrangements. The idea of first-rate management is to transmit the best via a set of movements supposed to stop and get rid of errors made at one-of-a-kind tiers of production. Before figuring out whether or not or no longer to speak an idea, at least one structure of manipulated motion needs to be taken. Rapid enlargement in the pharmaceutical enterprise over the previous few many years has coincided with the fast improvement of increasingly more state-of-the-art drug checkingout equipment. Providing a simple, clever method to deal with a difficult state of affairs is frequently very helpful. Therefore, it is vital to gain new clear techniques for these medicines. Next, we will have a seem at the motivations for the improvement of different latest drug checking out methods: Some pharmacopeias might also have barely off measurements for the pharmaceutical or prescription mix. The admission of an honestly rational framework for the medicinal drug in that can also be impeded by way of patent regulations. Third, combining medicines can make it hard to use evidence-based treatments. It may additionally be not possible to think about the use of scientific strategies to decide the genuine amount of medicinal drug in non-specialist fluids. 5. The persevered rational approach might also require high-priced solvents and reagents. It may additionally contain inefficient strategies for extracting and dividing food, neither of which are especially sturdy fits of be.

DIFFERENT METHODS OF ANALYSIS

Use the following techniques for dividing up and inspecting key components. Alternative World Tactics These methods are used to measure the model's absorbed or emitted electromagnetic radiation. Such strategies consist of ultraviolet-visible spectroscopy, infrared spectroscopy, nuclear magnetic resonance spectroscopy, electron spin resonance spectroscopy, X-ray photoelectron spectroscopy, and fluorescence spectroscopy. The methodology is primarily based on electro-coherence. The comparison of modern-day voltage or resistance as an excellent affiliation of the phase in diagram combination is what at the start attracted electro-realistic frameworks. Ampere-, coulomb-, and voltmeter-based fashions are all present. Methods for Chromatography is a method for isolating artificial chemical compounds by analyzing their sub-nuclear homes as they cross in discrete layers via a liquid or gas, or through a strong surface. There are many sorts of chromatography, inclusive of paper chromatography, analytical chromatography, excessive overall performance skinny layer chromatography (HPTLC), excessive overall performance liquid chromatography (HPLC), and fuel chromatography.

MATERIALS AND METHODS

Nortriptyline from Sura labs, Pregabalin from Sura labs, Water and Methanol for HPLC from LICHROSOLV (MERCK). Acetonitrile for HPLC from Merck, Phosphate buffer from Sura labs.

HPLC METHOD DEVELOPMENT: TRAILS

Preparation of standard solution:

Accurately weigh and transfer 10 mg of Nortriptyline and Pregabalin working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 0.2ml of the above Nortriptyline and 0.3ml of the Pregabalin stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure:

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

OPTIMIZED CHROMATOGRAPHIC CONDITIONS:

Instrument used: Waters HPLC with auto sampler and PDA Detector 996 model.
Temperature : Ambient
Column : Symmetry (C18) (150mm x 4.6mm, 5µm) Column
Buffe: Dissolve 6.8043 of potassium dihydrogen phosphate in 1000 ml HPLC water and adjust the pH 3.8 with diluted orthophosphoric acid. Filter and sonicate the solution by vacuum filtration and ultra sonication.
pH : 3.8
Mobile phase : Methanol: Phosphate Buffer (28:72%v/v)
Flow rate : 1ml/min
Wavelength : 252 nm
Injection volume : 20 µl
Run time : 8 min

VALIDATION

PREPARATION OF MOBILE PHASE:

Preparation of mobile phase:

Accurately measured 280 ml (28%) of Methanol, 720 ml of Phosphate buffer (72%) were mixed and degassed in digital ultra sonicater for 15 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation:

The Mobile phase was used as the diluent.

RESULTS AND DISCUSSION

Optimized Chromatogram (Standard)

Mobile phase ratio : Methanol: Phosphate Buffer (pH-3.8) (28:72v/v)
Column : Symmetry (C18) (150mm x 4.6mm, 5µm) Column
Columntemperature : Ambient
Wavelength : 252nm
Flow rate : 1.0ml/min
Injection volume : 20µl
Run time : 8minutes

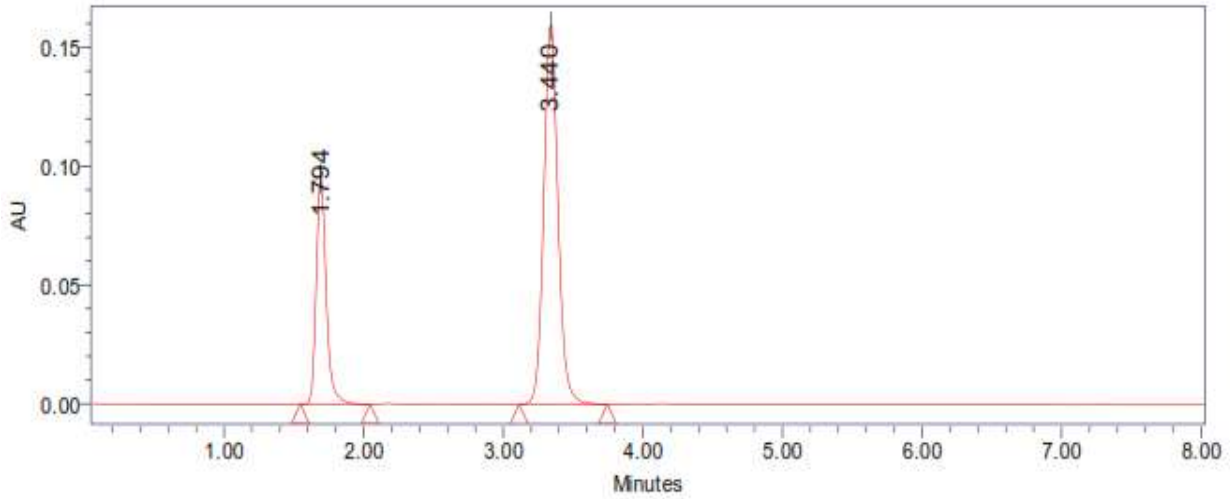

Fig.No.18: Optimized Chromatogram (Standard)

Table No.18: Optimized Chromatogram (Standard)

S.No.	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Nortriptyline	1.794	545265	7462	1.09	7564
2	Pregabalin	3.440	7768545	43652	1.12	8695

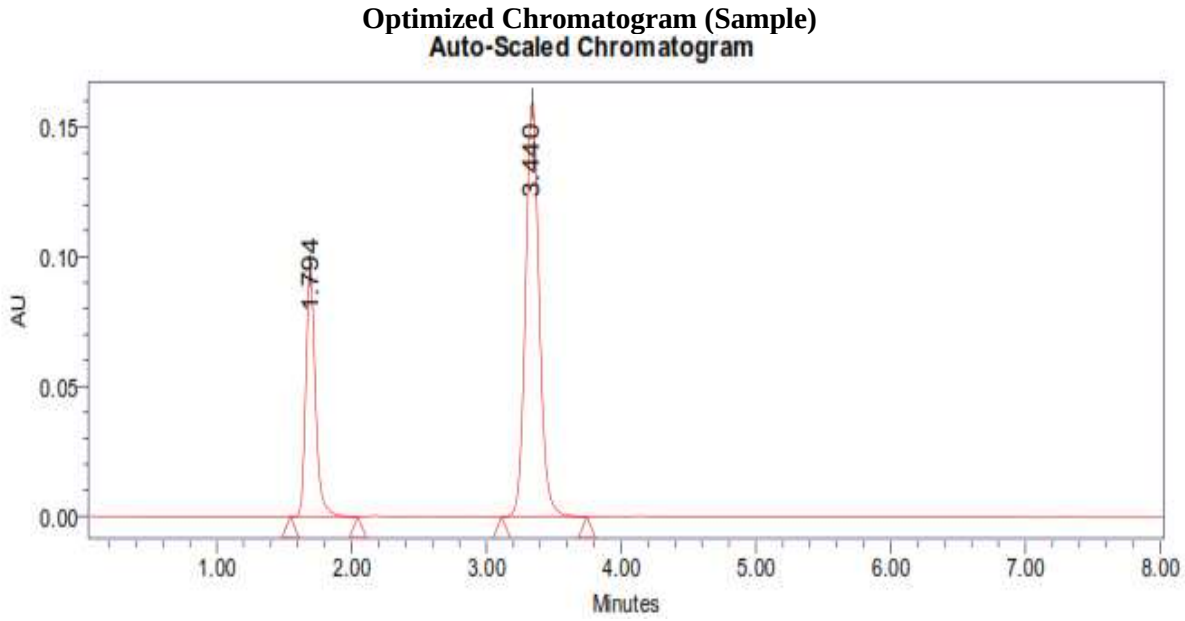

Fig.No.19: Optimized Chromatogram (Sample)

Table No. 19: Optimized Chromatogram (Sample)

S.No	Name	RT	Area	Height	USPTailing	USPPlateCount
1	Nortriptyline	1.794	558659	7584	1.10	7659
2	Pregabalin	3.440	7856985	44658	1.13	8743

AcceptanceCriteria:

- Theoretical plates must be not less than 2000.
- Tailing factor must be not less than 2.
- It wasfoundfromabovedatathatall the system suitability parameters for developed method were within the limit.

Assay (Standard):

Table-: Peak results forassaystandardof Nortriptyline

S.No.	Peak Name	RT	Area(µV*sec)	Height (µV)	USP PlateCount	USP Tailing
1	Nortriptyline	1.788	545698	7458	7595	1.09
2	Nortriptyline	1.792	548765	7469	7548	1.10
3	Nortriptyline	1.793	548965	7428	7563	1.09
4	Nortriptyline	1.788	548783	7495	7592	1.10
5	Nortriptyline	1.787	548752	7461	7543	1.09
Mean			548192.6			
Std.Dev.			1397.209			
%RSD			0.254876			

AcceptanceCriteria:

- %RSD of five different samplesolutionsshouldnotmorethan 2.
- The %RSD obtained is within the limit, hence the method is suitable.

Table: Peak results forassay standard of Pregabalin

S.No	Peak Name	RT	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Pregabalin	3.438	7785698	43652	8652	1.12
2	Pregabalin	3.446	7786354	43698	8674	1.13
3	Pregabalin	3.444	7786942	43587	8692	1.13
4	Pregabalin	3.465	7785464	43698	8649	1.12
5	Pregabalin	3.465	7785986	43568	8625	1.12
Mean			7786089			
Std.Dev.			581.3667			
%RSD			0.007467			

AcceptanceCriteria:

- %RSD of five different samplesolutionsshouldnotmorethan 2.
- The %RSD obtained is within the limit, hence the method is suitable.

Assay (Sample):

Table-: Peak results forAssaysampleof Nortriptyline

S.No	Name	RT	Area	Height	USPTailing	USPPlateCount	Injection
1	Nortriptyline	1.794	556985	75895	1.10	7698	1
2	Nortriptyline	1.791	558742	75468	1.10	7682	2
3	Nortriptyline	1.791	559683	75426	1.11	7649	3

Table : Peak results forAssaysampleof Pregabalin

S.No	Name	RT	Area	Height	USPTailing	USPPlateCount
1	Pregabalin	3.440	7856859	44586	1.14	8759
2	Pregabalin	3.442	7826594	44658	1.15	8726
3	Pregabalin	3.434	7854879	44859	1.14	8794

%ASSAY =

$$\frac{\text{Samplearea}}{\text{StandardareaDilutionofstandard}} \times \frac{\text{Weight ofstandardDilutionofsample}}{\text{Weight ofsample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight oftablet}}{\text{Labelclaim}} \times 100$$

The % purity of Nortriptyline and Pregabalinin pharmaceuticaldosageformwasfoundto be 100.154%

LINEARITYCHROMATOGRAPHIC DATA FOR LINEARITY STUDY:

NORTRIPTYLINE:

Concentration µg/ml	Average PeakArea
10	292985
15	430752
20	565265
25	693487
30	821584

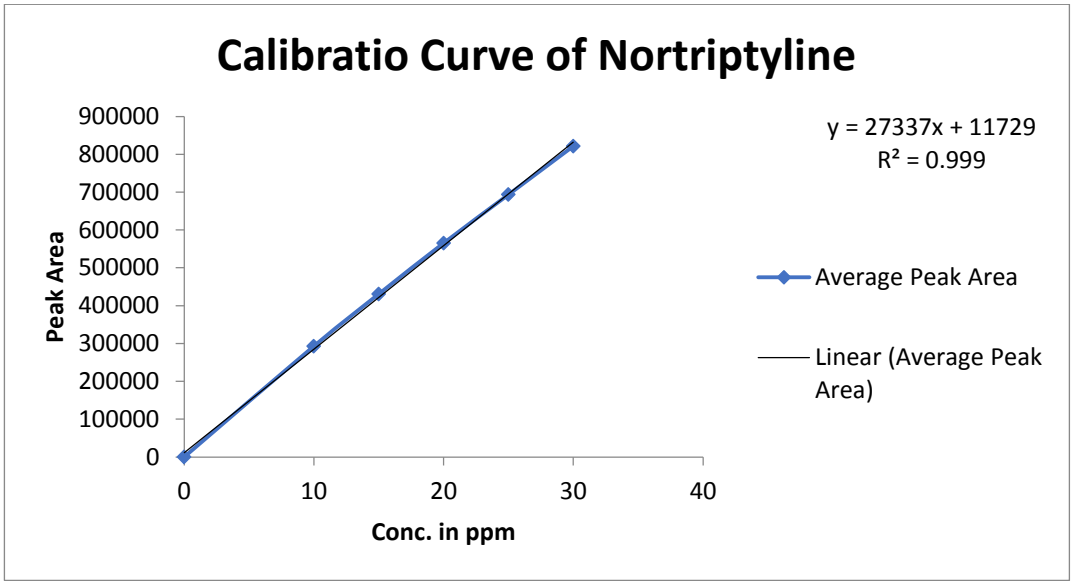

Fig: Chromatogramshowinglinearity level

PREGABALIN:

Concentration µg/ml	Average PeakArea
10	2828756
20	5485784
30	7999859
40	10656542
50	13085985

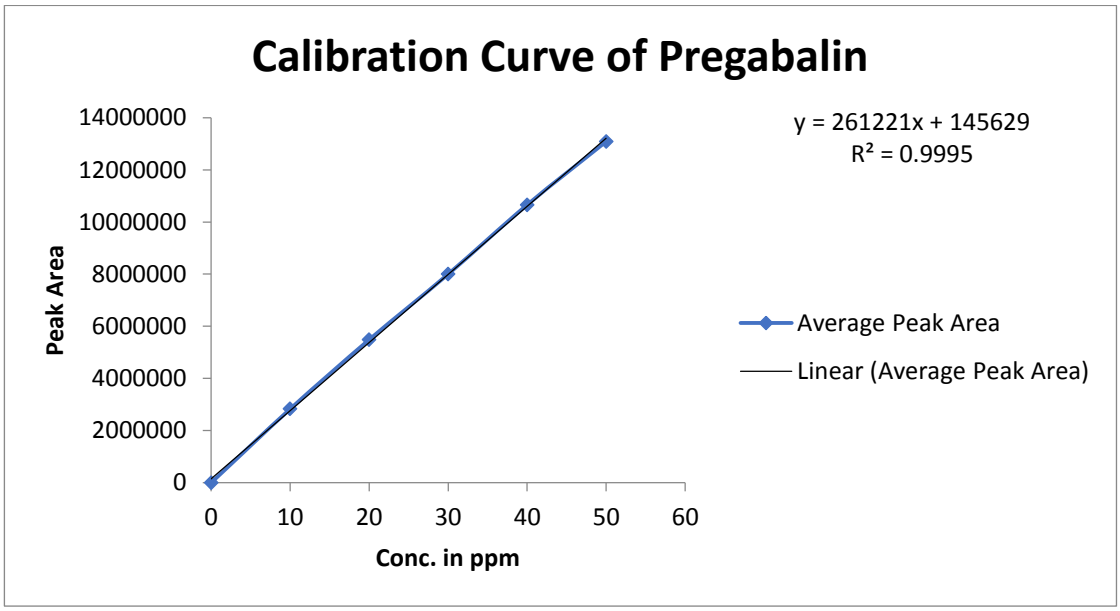

Fig: Chromatogramshowinglinearity level

REPEATABILITY

Table-: Results of Repeatability for Nortriptyline:

S. No.	Peak Name	Retention time	Area (µV*sec)	Height(µV)	USP Plate Count	USP Tailing
1	Nortriptyline	1.792	548698	7458	7569	1.10
2	Nortriptyline	1.791	548955	7485	7546	1.10
3	Nortriptyline	1.790	548745	7469	7592	1.09
4	Nortriptyline	1.790	549856	7463	7519	1.10
5	Nortriptyline	1.789	546587	7495	7535	1.09
Mean			548568.2			
Std.dev			1202.217			
%RSD			0.2191554			

AcceptanceCriteria:

- %RSD forsampleshould be NMT 2.
- The %RSD for the standardsolution is below 1, which is within the limits hence method is precise

Table : Results of Repeatability for Pregabalin:

S. No.	Peak Name	Retention time	Area (µV*sec)	Height (µV)	USP PlateCount	USP Tailing
1	Pregabalin	3.435	7768958	43659	8659	1.12
2	Pregabalin	3.428	7765984	43856	8647	1.13
3	Pregabalin	3.419	7785469	43658	8675	1.12
4	Pregabalin	3.414	7785498	43549	8652	1.12
5	Pregabalin	3.408	7769852	44526	8692	1.13
Mean			7775152			
Std.dev			9539.236			
%RSD			0.122689			

AcceptanceCriteria:

- %RSD forsampleshould be NMT 2.
- The %RSD for the standardsolution is below 1, which is within the limits hence method is precise.

Intermediate precision:

Table-: Results ofIntermediate precision day1forNortriptyline

S.No.	Peak Name	RT	Area(µV*sec)	Height (µV)	USP Platecount	USP Tailing
1	Nortriptyline	1.787	556985	75986	7695	1.11
2	Nortriptyline	1.789	558649	75986	7642	1.12
3	Nortriptyline	1.789	557847	75689	7683	1.12
Mean			557827			
Std.Dev.			832.1803			
%RSD			0.149183			

AcceptanceCriteria:

%RSD of three different samplesolutionsshouldnotmorethan 2.

Table: Results ofIntermediate precision day1forPregabalin

S.No.	Peak Name	RT	Area(µV*sec)	Height (µV)	USP Platecount	USP Tailing
1	Pregabalin	3.482	7856982	44586	8758	1.13
2	Pregabalin	3.477	7845285	44758	8769	1.14
3	Pregabalin	3.477	7854633	44986	8728	1.13
Mean			7852300			
Std.Dev.			6187.659			
%RSD			0.078801			

AcceptanceCriteria:

- %RSD of three different samplesolutionsshouldnotmorethan 2.

Table-: Results of Intermediate precision Day 2 forNortriptyline

S.No.	Peak Name	RT	Area(µV*sec)	Height (µV)	USP Platecount	USP Tailing
1	Nortriptyline	1.790	536598	7365	7459	1.08
2	Nortriptyline	1.789	534875	7358	7436	1.07
3	Nortriptyline	1.793	534698	7349	7482	1.08
Mean			535390.3			
Std.Dev.			1049.608			
%RSD			0.196045			

AcceptanceCriteria:

%RSD of three different samplesolutionsshouldnotmorethan 2.

Table: Results of Intermediate precision Day 2 forPregabalin

S.No.	Peak Name	RT	Area(µV*sec)	Height (µV)	USP Platecount	USP Tailing
1	Pregabalin	3.474	7698521	42568	8582	1.11
2	Pregabalin	3.473	7685985	42698	8546	1.10
3	Pregabalin	3.478	7645897	42365	8574	1.10
Mean			7676801			
Std.Dev.			27487.83			
%RSD			0.358064			

AcceptanceCriteria:

- %RSD of three different samplesolutionsshouldnotmorethan 2.

ACCURACY:

Table-: The accuracy results for Nortriptyline

%Concentration (at Specification Level)	Area	AmountAdded (ppm)	AmountFound (ppm)	% Recovery	Mean Recovery
50%	286080.7	10.035	10	100.350%	100.291%
100%	561215	20.100	20	100.500%	
150%	833959.7	30.077	30	100.023%	

Table : The accuracy results for Pregabalin

%Concentration (at Specification Level)	Area	AmountAdded (ppm)	AmountFound (ppm)	% Recovery	Mean Recovery
50%	408328	15	15.074	100.493%	100.163%
100%	798306.3	30	30.003	100.010%	
150%	1189915	45	44.994	99.986%	

Robustness

Table-: Results for Robustness

Results for Robustness -Nortriptyline

Parameter used for sample analysis	PeakArea	Retention Time	Theoretical plates	Tailing factor
ActualFlowrateof 0.9mL/min	545265	1.794	7564	1.09
Less Flowrateof 0.8mL/min	625486	1.867	7856	1.13
MoreFlowrateof 1.0mL/min MoreFlowrateof 0.9mL/min	526548	1.744	7425	1.12

Less organicphase (about 5 % decrease in organicphase)	536548	1.831	7265	1.06
Moreorganicphase (about 5 % Increase in organicphase)	514875	1.874	7169	1.08

Table : Results for Robustness-Pregabalin

Parameter used for sample analysis	PeakArea	Retention Time	Theoretical plates	Tailing factor
ActualFlowrateof 0.9mL/min	7768545	3.440	8695	1.12
Less Flowrate of 0.8mL/min	7985695	3.721	8948	1.13
MoreFlowrateof 1.0mL/min	7458642	3.097	8452	1.12
Less organicphase (about 5 % decrease in organicphase)	7685421	6.242	8365	1.10
Moreorganicphase (about 5 % Increase in organicphase)	7569864	2.402	8254	1.09

AcceptanceCriteria:
The Tailing factor should be less than 2.0 and the number of theoretical plates (N) should be morethan 2000.

CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Nortriptyline and Pregabalin in bulk drug and pharmaceutical dosage forms.

Nortriptyline (hydrochloride) is soluble in organic solvents such as ethanol, DMSO, and dimethyl formamide (DMF) methylene chloride, which should be purged with an inert gas. The solubility of Nortriptyline (hydrochloride) in ethanol is approximately 15 mg/ml and approximately 30 mg/ml in DMSO and DMF, slightly soluble in methanol, chloroform and water, sparingly soluble in water; soluble in alcohol and in dichloromethane. Pregabalin is freely soluble in water and both basic and acidic solutions, sparingly soluble in organic solvents such as ethanol, DMSO, and dimethyl formamide.

Methanol: Phosphate Buffer (pH-3.8) (28:72v/v) 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 inTablesfor 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 Nortriptyline and Pregabalin in bulk drug and in Pharmaceutical dosage forms.

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