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Research

Method development and validation for the simultaneous estimation of nortriptyline and pregabalin in bulk and marketed pharmaceutical dosage form by using reverse phase-hplc method

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Abstract

A simple, reproducible and efficient reverse phase high performance liquid chromatographic method was developed for simultaneous determination of Nortriptyline and Pregabalin in pure form and marketed combined pharmaceutical dosage forms. A column having Symmetry (C18) (150mm x 4.6mm, 5µm) in isocratic mode with mobile phase containing Methanol: Phosphate Buffer (pH-3.8) (28:72v/v) was used. The flow rate was 1.0 ml/min and effluent was monitored at 252 nm. The retention time (min) and linearity range (ppm) for Nortriptyline and Pregabalin were (1.794, 3.440min) and (10-30, 10-50), respectively. The method has been validated for linearity, accuracy and precision, robustness and limit of detection and limit of quantitation. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 0.86µg/ml and 2.58µg/ml for Nortriptyline and 1.28µg/ml 3.84µg/ml for Pregabalin respectively. The developed method was found to be accurate, precise and selective for simultaneous determination of Nortriptyline and Pregabalin in bulk form and marketed combined pharmaceutical dosage forms.

Keywords: Nortriptyline and Pregabalin, RP-HPLC, Validation, Accuracy, Precision.

INTRODUCTION

Analysis may be defined as the science and art of determining the composition of materials in terms of the elements or compounds contained in them. In fact, analytical chemistry is the science of chemical identification and determination of the composition (atomic, molecular) of substances, materials and their chemical structure.

Chemical compounds and metallic ions are the basic building blocks of all biological structures and processes which are the basis of life. Some of these naturally occurring compounds and ions (endogenous species) are present only in very small amounts in specific regions of the body, while others such as peptides, proteins, carbohydrates, lipids and nucleic acids are found in all parts of the body. The main object of analytical chemistry is to develop scientifically substantiated methods that allow the qualitative and quantitative evaluation of materials with certain accuracy. Analytical chemistry derives its principles from various branches of science

like chemistry, physics, microbiology, nuclear science and electronics. This method provides information about the relative amount of one or more of these components.[1]

Every country has legislation on bulk drugs and their pharmaceutical formulations that sets standards and obligatory quality indices for them. These regulations are presented in separate articles relating to individual drugs and are published in the form of book called "Pharmacopoeia" (e.g. IP, USP, and BP). Quantitative chemical analysis is an important tool to assure that the raw material used and the intermediate products meet the required specifications. Every year number of drugs is introduced into the market. Also quality is important in every product or service, but it is vital in medicines as it involves life.

There is a time lag from the date of introduction of a drug into the market to the date of its inclusion in pharmacopoeias. This happens because of the possible uncertainties in the continuous and wider usage of these drugs, report of new toxicities and development of patient resistance and introduction of better drugs by the competitors. Under these conditions standard and analytical procedures for these drugs may not be available in Pharmacopoeias. In instrumental analysis, a physical property of the substance is measured to determine its chemical composition. Pharmaceutical analysis comprises those procedures necessary to determine the identity, strength, quality and purity of substances of therapeutic importance.[2]

Pharmaceutical analysis deals not only with medicaments (drugs and their formulations) but also with their precursors i.e. with the raw material on which degree of purity and quality of medicament depends. The quality of the drug is determined after establishing its authenticity by testing its purity and the quality of pure substance in the drug and its formulations.

Quality control is a concept which strives to produce a perfect product by series of measures designed to prevent and eliminate errors at different stages of production. The decision to release or reject a product is based on one or more type of control action. With the growth of pharmaceutical industry during last several years, there has been rapid progress in the field of pharmaceutical analysis involving complex instrumentation. Providing simple analytical procedure for complex formulation is a matter of most importance. So, it becomes necessary to develop new analytical methods for such drugs. In brief the reasons for the development of newer methods of drugs analysis are:

1. The drug or drug combination may not be official in any pharmacopoeias.
2. A proper analytical procedure for the drug may not be available in the literature due to Patent regulations.
3. Analytical methods for a drug in combination with other drugs may not be available.
4. Analytical methods for the quantitation of the drug in biological fluids may not be available.
5. The existing analytical procedures may require expensive reagents and solvents. It may also involve cumbersome extraction and separation procedures and these may not be reliable.[1 2]

Different methods of analysis

The following techniques are available for separation and analysis of components of interest.

Spectral methods

The spectral techniques are used to measure electromagnetic radiation which is either absorbed or emitted by the sample. E.g. UV-Visible spectroscopy, IR spectroscopy, NMR, ESR spectroscopy, Flame photometry, Fluorimetry.2

Electro analytical methods

Electro analytical methods involved in the measurement of current voltage or resistanceas a property of concentration of the component in solution mixture. E.g. Potentiometry, Conductometry, Amperometry.[2]

Chromatographic methods

Chromatography is a technique in which chemicals in solutions travel down columns or over surface by means of liquids or gases and are separated from each other due to their molecular characteristics. E.g. Paper chromatography, thin layer chromatography (TLC), High performance thin layer chromatography (HPTLC), High performance liquid chromatography (HPLC), Gas chromatography (GC).[2]

Miscellaneous Techniques

Mass Spectrometry, Thermal Analysis.

Hyphenated Techniques

GC-MS (Gas Chromatography – Mass Spectrometry), LC-MS (Liquid Chromatography – Mass Spectrometry), ICP-MS (Inductivity Coupled Plasma- Mass Spectrometry), GC-IR (Gas Chromatography – Infrared Spectroscopy), MS-MS (Mass Spectrometry – Mass Spectrometry). Analytical techniques that are generally used for drug analysis also include biological and microbiological methods, radioactive methods and physical methods etc.

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
Buffer	:	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
Column temperature	: Ambient
Wavelength	: 252nm
Flow rate	: 1.0ml/min
Injection volume	: 20µl
Run time	: 8minutes

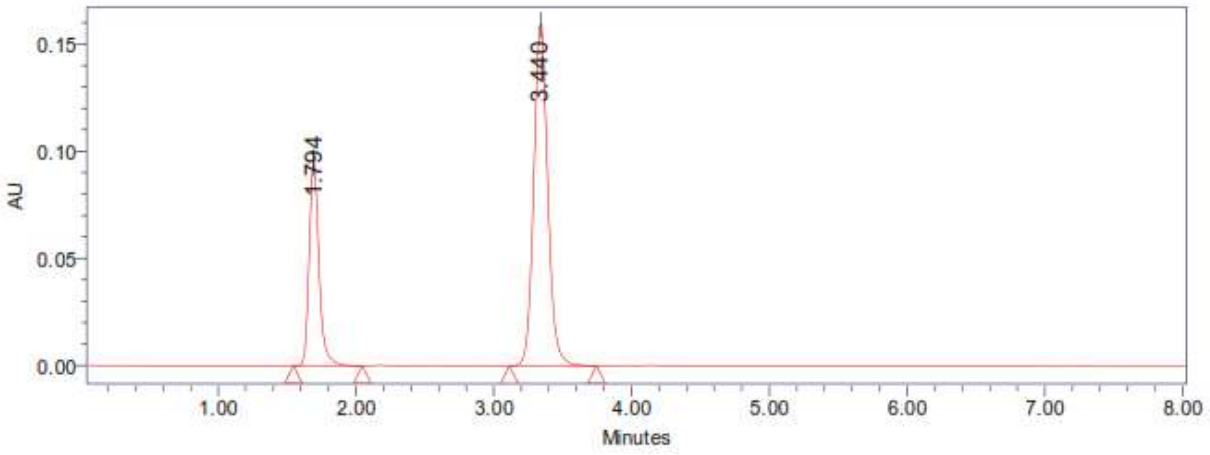

Fig 1: Optimized Chromatogram (Standard)

Table 1: 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

Optimized Chromatogram (Sample)

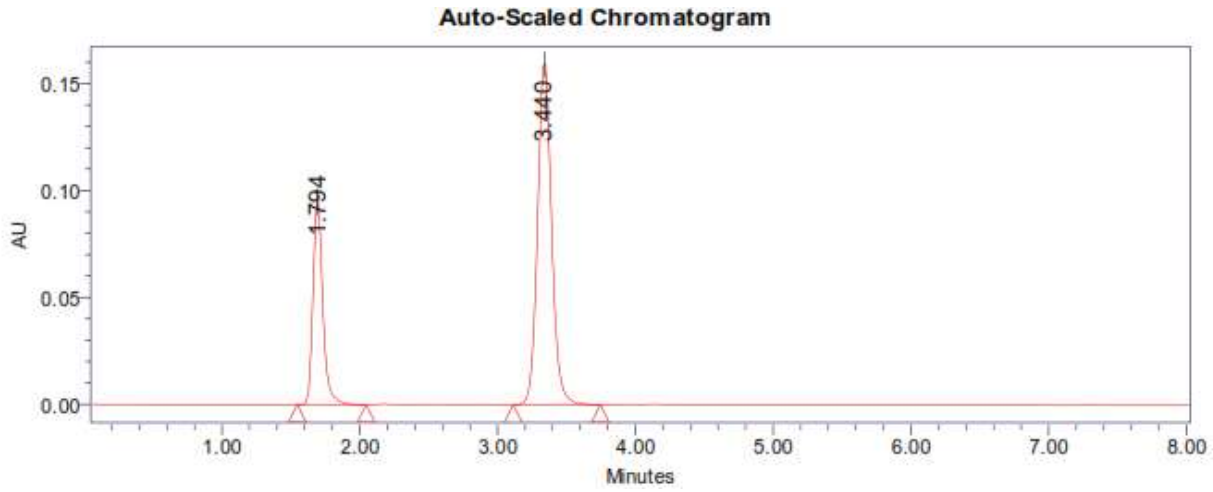

Fig 2: Optimized Chromatogram (Sample)

Table 2: Optimized Chromatogram (Sample)

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Nortriptyline	1.794	558659	7584	1.10	7659
2	Pregabalin	3.440	7856985	44658	1.13	8743

- Theoretical plates must be not less than 2000.
- Tailing factor must be not less than 2.
- It was found from above data that all the system suitability parameters for developed method were within the limit.

Assay (Standard)

Table 3: Peak results for assay standard of Nortriptyline

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	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			

- *%RSD of five different sample solutions should not more than 2.*
- *The %RSD obtained is within the limit, hence the method is suitable.*

Table 4: Peak results for assay 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			

- *%RSD of five different sample solutions should not more than 2.*
- *The %RSD obtained is within the limit, hence the method is suitable.*

Assay (Sample)

Table 5: Peak results for Assay sample of Nortriptyline

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	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 6: Peak results for Assay sample of Pregabalin

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count
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

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

The % purity of Nortriptyline and Pregabalin in pharmaceutical dosage form was found to be 100.154%

Linearity
Chromatographic data for linearity study
Nortriptyline

Concentration µg/ml	Average Peak Area
10	292985

15	430752
20	565265
25	693487
30	821584

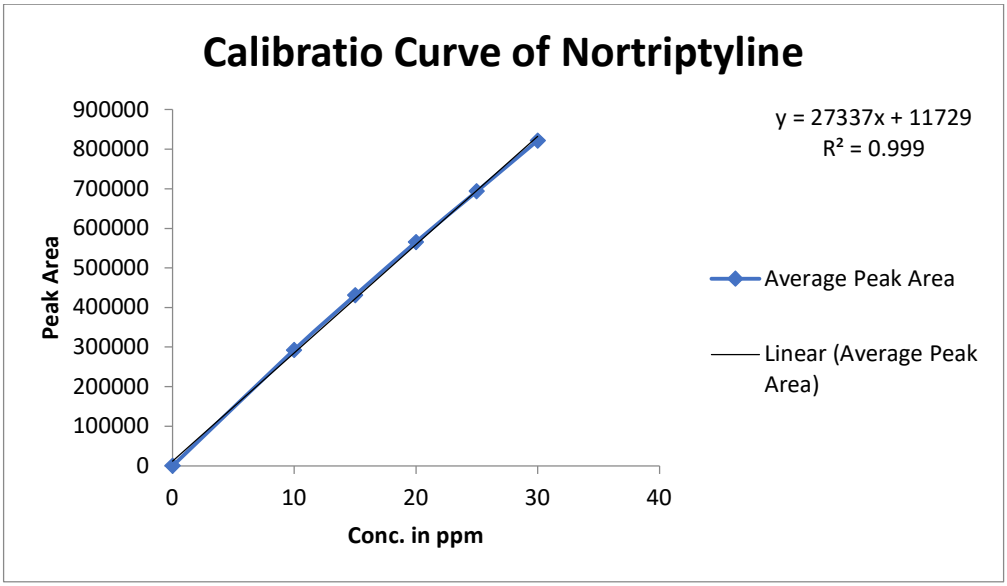

Fig 3: Chromatogram showing linearity level

PREGABALIN

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

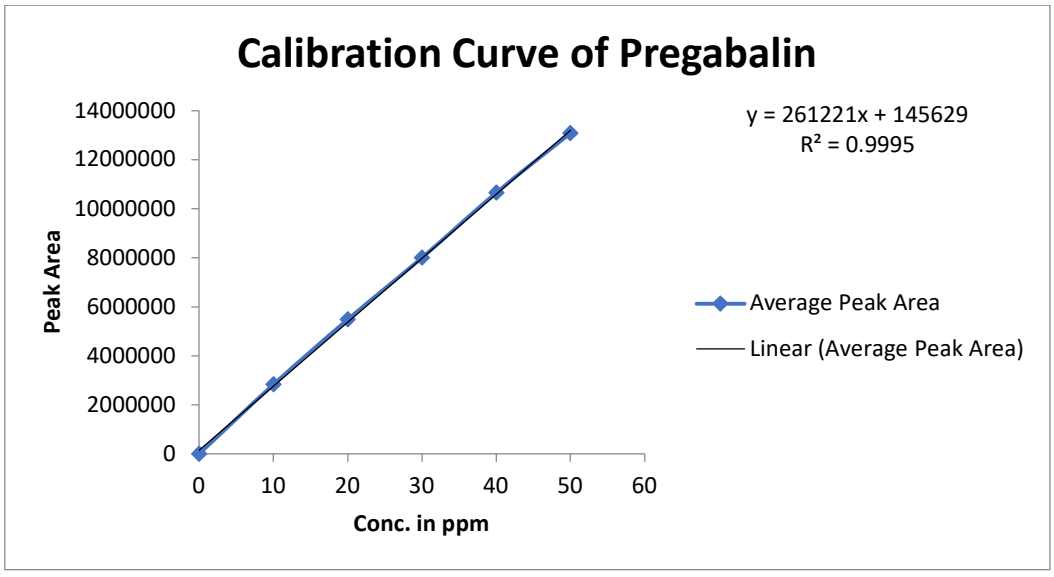

Fig 4: Chromatogram showing linearity level

REPEATABILITY

Table 7: 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			

- *%RSD for sample should be NMT 2.*
- *The %RSD for the standard solution is below 1, which is within the limits hence method is precise.*

Table 8: Results of Repeatability for Pregabalin

S. No.	Peak Name	Retention time	Area (µV*sec)	Height (µV)	USP Plate Count	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			

- *%RSD for sample should be NMT 2.*
- *The %RSD for the standard solution is below 1, which is within the limits hence method is precise.*

Intermediate precision

Table 9: Results of Intermediate precision day1 for Nortriptyline

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	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			

%RSD of three different sample solutions should not more than 2.

Table 10: Results of Intermediate precision day1 for Pregabalin

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	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			

- *%RSD of three different sample solutions should not more than 2.*

Table 11: Results of Intermediate precision Day 2 for Nortriptyline

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	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			

%RSD of three different sample solutions should not more than 2.

Table 12: Results of Intermediate precision Day 2 for Pregabalin

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	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			

- *%RSD of three different sample solutions should not more than 2.*

Accuracy

Table 13: The accuracy results for Nortriptyline

%Concentration (at Specification Level)	Area	Amount Added (ppm)	Amount Found (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 14: The accuracy results for Pregabalin

%Concentration (at Specification Level)	Area	Amount Added (ppm)	Amount Found (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 15: Results for Robustness

Results for Robustness -Nortriptyline

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 0.9mL/min	545265	1.794	7564	1.09
Less Flow rate of 0.8mL/min	625486	1.867	7856	1.13
More Flow rate of 1.0mL/min More Flow rate of 0.9mL/min	526548	1.744	7425	1.12
Less organic phase (about 5 % decrease in organic phase)	536548	1.831	7265	1.06

More organic phase (about 5 % Increase in organic phase)	514875	1.874	7169	1.08

Table 16: Results for Robustness-Pregabalin

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 0.9mL/min	7768545	3.440	8695	1.12
Less Flow rate of 0.8mL/min	7985695	3.721	8948	1.13
More Flow rate of 1.0mL/min	7458642	3.097	8452	1.12
Less organic phase (about 5 % decrease in organic phase)	7685421	6.242	8365	1.10
More organic phase (about 5 % Increase in organic phase)	7569864	2.402	8254	1.09

The Tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 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 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 Nortriptyline and Pregabalin in bulk drug and in Pharmaceutical dosage forms.

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