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RESEARCH ARTICLE

Stability Indicating RP-HPLC Method Development and Validation for Simultaneous Estimation of Pregabalin and Nortriptyline in Tablet

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ABSTRACT

Stability indicating a RP-HPLC method for simultaneous estimation of Nortriptyline Hydrochloride and Pregabalin in their Combined Dosage Form has been developed. A reverse phase high performance liquid chromatographic method was developed for the simultaneous estimation of Nortriptyline Hydrochloride and Pregabalin in their Combined Dosage Form has been developed. The separation was achieved by LC- 20 AT C_{18} (250mm x 4.6 mm x 2.6 µm) column and Phosphate Buffer (pH 5.0): Methanol (70:30, V/V) as mobile phase, at a flow rate of 1ml/min. Detection was carried out at 210 nm. The retention time of Nortriptyline Hydrochloride and Pregabalin were found to be 3.203 min and 5.400 min, respectively. The method has been validated for linearity, accuracy, and precision. Linearity observed for Nortriptyline Hydrochloride 5-15 µg/ml and for Pregabalin 37.5-112.5 µg/ml. Developed method was found to be accurate, precise and rapid for simultaneous estimation of Nortriptyline Hydrochloride and Pregabalin in their Combined Dosage Form.

KEYWORDS

Nortriptyline Hydrochloride, Pregabalin, Stability indicating RP-HPLC Method

INTRODUCTION

Nortriptyline is methyl (3-{tricyclo[9.4.0.0^{3,8}] pentadeca-1(15),3,5,7,11,13-hexaen-2-ylidene} propyl) amine a tricyclic antidepressant agent used for short-term treatment of various forms of depression. Nortriptyline blocks the norepinephrine presynaptic receptors, thereby blocking the reuptake of this neurotransmitter and raising the concentration in the synaptic cleft in the CNS. Nortriptyline also binds to alpha-adrenergic, histaminergic and cholinergic receptors.

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Long-term treatment with nortriptyline produces a down-regulation of adrenergic receptors due to the increased stimulation of these receptors. Sparingly soluble in methanol; practically insoluble in most organic solvents [1, 2]. It is official in IP, BP, and USP[5, 6, 7].

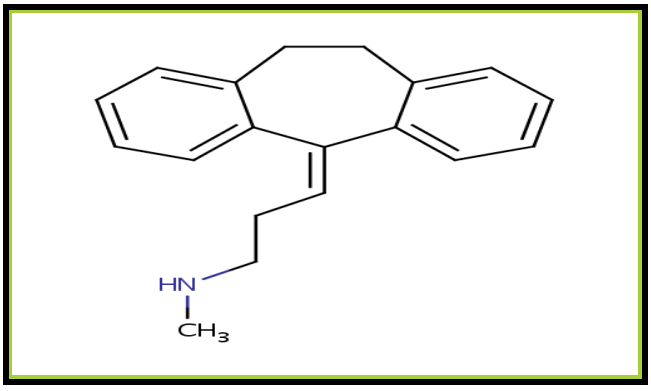

Figure No.:-1 Structure of Pregabalin

- Pregabalin is a (3S)-3-(aminomethyl)-5-methyl hexanoic acid binds with high affinity to the alpha2-delta site (an auxiliary subunit of voltage-gated calcium channels) in central nervous system tissues. Although the mechanism of action of pregabalin is unknown, results with genetically modified mice and with compounds structurally related to pregabalin (such as gabapentin) suggest that binding to the alpha2-delta subunit may be involved in pregabalin's antinociceptive and antiseizure effects in animal models. In vitro, pregabalin reduces the calcium-dependent release of several neurotransmitters, possibly by modulation of calcium channel function. Freely soluble in water and both basic and acidic solutions3, 4. It is Official in IP8. The proposed method was validated with respect to specificity, linearity, accuracy, precision and robustness. In addition, stress testing of the drug was also conducted, as required by the International Conference on Harmonization (ICH) 9 to support the suitability of the method.

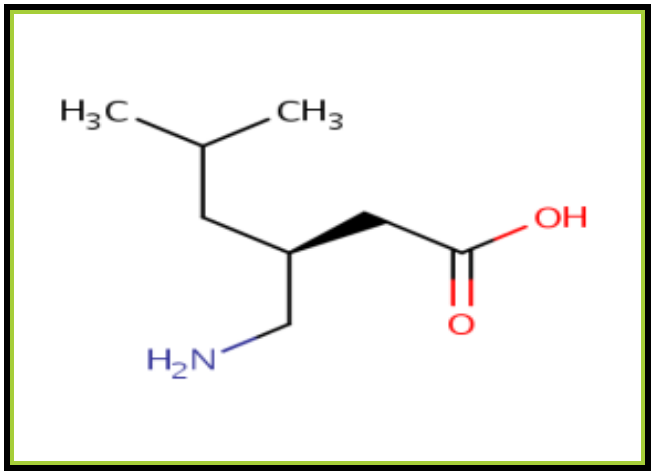

Figure 2: Structure of Pregabalin

MATERIALS AND METHOD

Instrumentation:

The chromatography was performed on a Shimadzu HPLC instrument (LC-20AT) equipped With standard PDA 600 UV Detector and Spinchrom software, BDS hypersil C18 column (250mm, 4.6mm, and 5µm**)** thermal scientific was used as stationary phase Injector, 20µL fixed loop, Electronic analytical balance Corning volumetric flasks and pipettes were used in the study.

CHEMICALS AND SOLVENTS:

Pregabalin and Nortriptyline standards and its marketed formulation NORTIPAN (containing Pregabalin 75 mg & Nortriptyline10mg) were kindly supplied as a gift sample from Medley Pharmaceutical Pvt. Ltd., Pregabalin was procured from Intas Pharma & Nortriptyline was Procured from Unison Pharma. HPLC grades Acetonitrile, Methanol, triple distilled water (Finar Chemicals Ltd., Mumbai, India) were used and AR grade Hydrochloric Acid, Sodium Hydroxide, Hydrogen Peroxide (Finar Chemicals Ltd., Mumbai, India), Orthophosphoric acid, Triethylamine and Potassium dihydrogen phosphate (Merck India Ltd.) were used. Whatman Filter paper no. 41 (Whatman International Ltd., England) was used in the study.

Preparation of standard solutions:

(A) Pregabalin standard stock solution :(750 µg/mL)

A 75 mg of Pregabalin was weighed and transferred to a 100 ml volumetric flask. Volume was made up to the mark with methanol.

(B) Nortriptyline Hydrochloride standard stock solution :(100 µg/mL)

A 10 mg of Nortriptyline Hydrochloride was weighed and transferred to a 100 ml volumetric flask. Volume was made up to the mark with methanol.

(C) Preparation of standard solution of binary mixtures of Pregabalin (75 µg/mL) and Nortriptyline Hydrochloride :(10 µg/mL)

Take 1 ml from the Pregabalin stock solution and 1ml from Nortriptyline Hydrochloride stock solution and transferred to 10 ml volumetric flask and volume made up to the mark by the mobile phase which was used in particular trials.

Analytical Method Development:-

To optimize the HPLC parameters, several mobile phase compositions were tried. Satisfactory results were obtained from given chromatographic condition for Pregabalin and Nortriptyline.

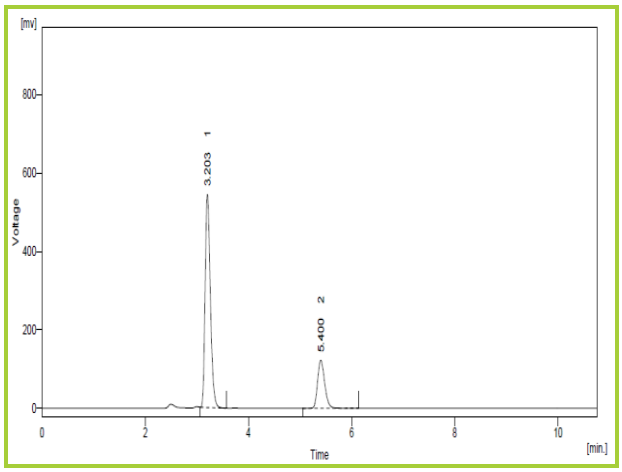

Figure 3: Chromatogram of Pregabalin and Nortriptyline Hydrochloride in Buffer (pH 5.0): Methanol (70:30: v/v) (Flow rate-1.0 ml/min)

Table 1: Optimized Chromatographic Conditions

Parameters	Conditions
Mobile Phase	Buffer (ph 5.0): Methanol (70:30: v/v)
Stationary Phase	BDS hypersil C18 column (250mm, 4.6mm, and 5µm)
Flow rate (ml/min)	1 ml/min
Run Time (min)	8 min
Injection Volume (µL)	20 µL
Detection Wavelength(nm)	210 nm
Retension Time (min)	Nortriptyline: 3.203 Pregabalin: 5.400

Analytical Method Validation:-

The developed chromatographic method was validated as per ICH guideline for following parameters.

System Suitability:-

As per USP-24, system suitability tests were carried out on freshly prepared standard stock solution of Pregabalin and Nortriptyline of both drugs under optimized chromatographic condition and parameters were studied to evaluate the suitability of the system. Results are shown in Table: 2.

Table 2: System Suitability Studies

Parameter	Pregabalin	Nortriptyline	Acceptance Criteria
Theoretical Plate	8101	8136	NLT 2000
Tailing Factor	1.310	1.333	NMT 2
Ressolution	12.142		NLT 2

Linearity and Range:

The drug response was linear over the concentration range between 37.5-112.5 µg/ml for Nortriptyline and 5-15µg/ml for Pregabalin. The results are shown in Figure: 4, 5 & 6 and Table 3.

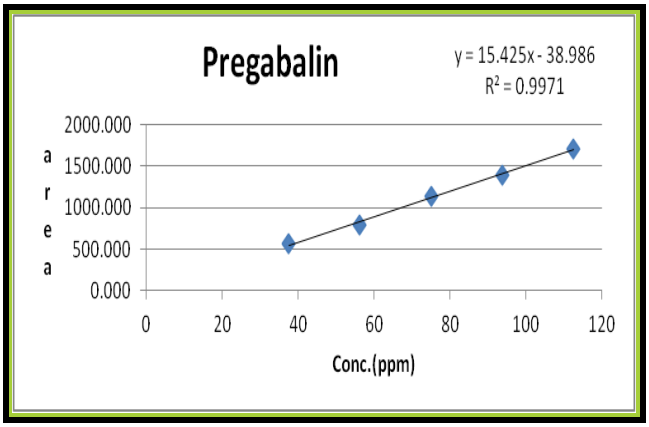

Figure 4: Calibration curve of Pregabalin

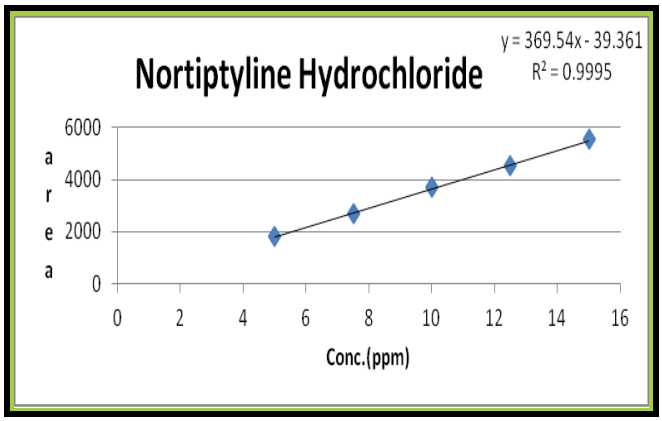

Figure 5: Calibration curve of Nortriptyline

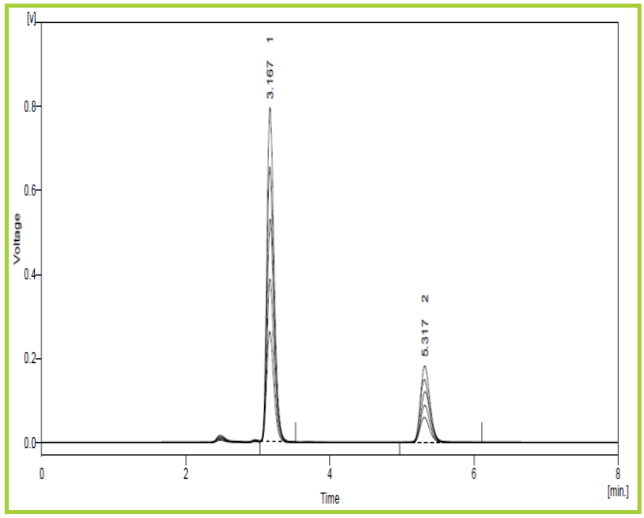

Figure 6: Overlay chromatogram of different concentrations of binary mixtures of Pregabalin and Nortriptyline Hydrochloride

Accuracy:

Good recoveries of Pregabalin and Nortriptyline were obtained at various added concentrations by spiking standards like 80 %, 100 % and 120 %. Results are shown in Table 3.

Precision:

The results of the repeatability, intra-day, and inter-day precision experiments are shown respectively as given in Table 3. The developed method was found to be precise as the % RSD were < 2%.

Robustness:

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small but deliberate variations in the analytical procedure parameters [pH (±0.2), Flow rate (±0.2 ml) and proportion of mobile phase (±2.0 v/v]. The standard deviation of the peak is calculated for each parameter and the %RSD was found to be less than 2%. Results are shown in Table 3.

Degradation Study:

The drug content was employed for acidic, alkaline, and oxidant media and also for thermal and photolytic stress conditions. After the degradation treatments were completed, the stress content solutions were allowed to equilibrate to room temperature and diluted with diluent to attain Pregabalin 75 PPM and Nortriptyline 10 PPM concentration, 20µl were injected into the system and the chromatograms were recorded to assess the stability of the sample. Specific degradation conditions described as following.

Acidic Degradation Condition:

Acid decomposition studies were performed by Refluxing 1ml of the stock solution was transferred into 10 ml of volumetric flask. Two ml of 0.1 N HCl solutions was added and mixed well and put for 2hrs at 70 ºC 250 ml Round bottom flask.

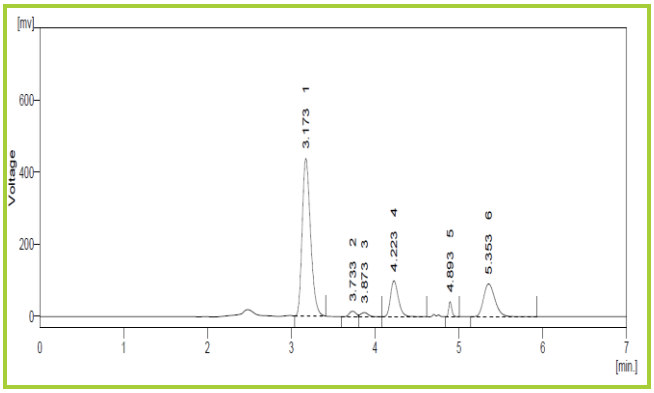

Figure 7: Pregabalin and Nortriptyline Hydrochloride Acid Degradation Sample

Base Degradation Condition:

Base decomposition studies were performed by refluxing 1ml of the stock solution was transferred into 10 ml of volumetric flask. Two ml of 0.1 N NaOH solutions was added and mixed well and put for 3 hrs of different intervals at 70 ºC 250 ml Round bottom flask.

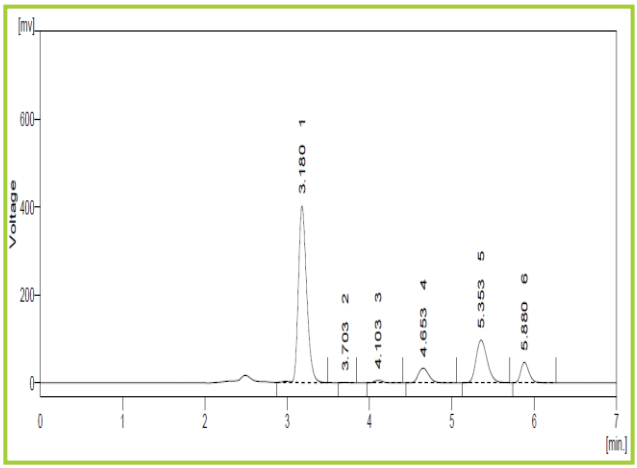

Figure 8: Pregabalin and Nortriptyline Hydrochloride Base Degradation Sample

Oxidative Degradation Condition:–

Oxidative decomposition studies were performed by refluxing 1ml of the stock solution was transferred into 10 ml of volumetric flask. Two ml of 3% H_2O_2 solutions was added and mixed well and put for 2hrs at 70 ºC 250 ml Round bottom flask.

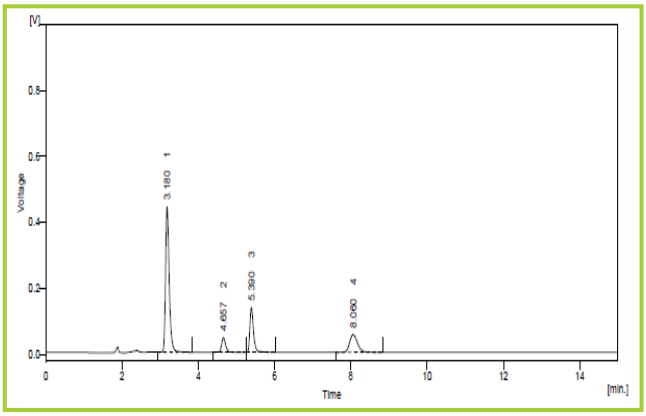

Figure 9: Pregabalin and Nortriptyline Hydrochloride Oxidation Degradation sample

Thermal Degradation Condition:

Thermal Degradation studies were performed 1 ml of stock solution was transferred into 10 ml of volumetric flask. The volumetric flask was stored in an oven at 105°C for 4 hrs.

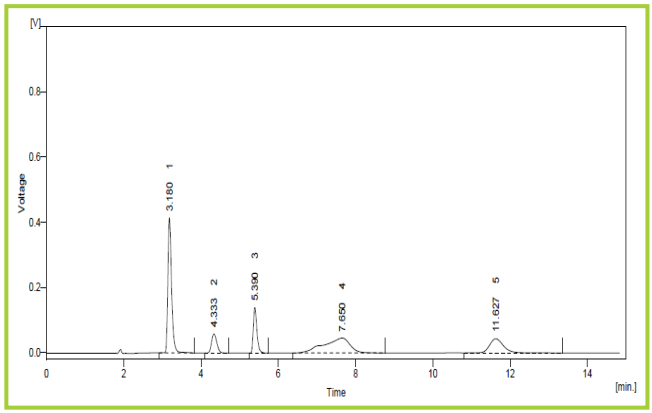

Figure 10: Nortriptyline Hydrochloride and Pregabalin Thermal Degradation sample

Photolytic Degradation Condition:-

Photo Degradation studies were performed 1ml of the stock solution was transferred into 10 ml of volumetric flask. The volumetric flask was kept in presence of UV-Chamber for 24 hrs.

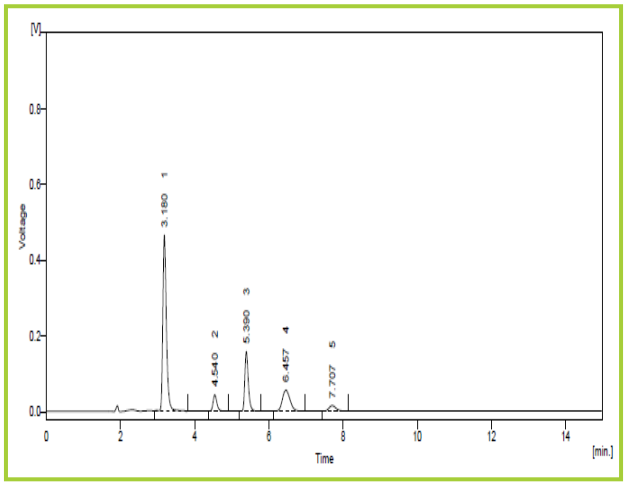

Figure 11: Nortriptyline Hydrochloride and Pregabalin Photo Degradation sample

RESULTS AND DISCUSSION

Validation Parameters:- The method was validated in compliance with ICH guidelines[9].

Force Degradation Studies:- In the present investigation of the Pregabalin and Nortriptyline were subjected to its stability studies as per ICH guideline[9]. The results of the forced degradation study of Pregabalin and Nortriptyline are summarized in Table 4 & 5.

Table 3: Regression analysis data and summary of validation parameter

Parameters		Pregabalin	Nortriptyline
Linearity range(n=3) (µg/ml)		37.5-112.5	5-15
regression equation(r^2)		Y = 5.784x - 38.98	Y=739.0x - 39.36
co-relation coefficient		0.997	0.999
Lod(µg/ml)		6.118	0.348
Loq(µg/ml)		18.539	1.053
Recovery%		99.72-100.02	99.87-100.17
Repeatability(% rsd nmt 2)		1.038	0.497
intra-day (n=3) precision (% rsd nmt 2)		0.380-0.418	266-0.747
Inter-day (n=3) precision (% rsd nmt 2)		0.812-1.064	0.988-1.288
Robbustness	Ph (± 0.2)	(-)1.250,(+)1.815	(-)1.506,(+)0.937
	Flow rate (± 0.2 ml)	(-)1.380,(+)1.645	(-)0.597,(+)0.755
	Mobile phase ratio (± 2 ml)	(-)1.380,(+)1.645	(-)0.630,(+)0.996
Assay		99.343± 1.162	100.803± 1.489

Table 4: Results of forced degradation study of Pregabalin

Pregabalin				
Parameter	Standard		Sample	
	Area	%Degradation	Area	%Degradation
Acid	863.25	23.66	855.87	24.31
base	895.39	20.82	906.93	19.80
Oxidation	873.54	22.75	893.35	21.00
Photo	1000.02	11.56	1014.78	10.26
Thermal	896.52	20.73	899.87	20.42

Table 5: Results of forced degradation study of Nortriptyline

Nortriptyline Hydrochloride				
Parameter	Standard		Sample	
	Area	%Degradation	Area	%Degradation
Acid	3040.17	20.16	3030.06	20.43
base	2686.10	29.46	2824.84	25.82
oxidation	2902.01	23.79	2932.67	22.98
Photo	3122.29	18.00	3085.23	18.98
Thermal	2728.08	29.36	2765.01	27.39

CONCLUSION

The HPLC method developed for the analysis of Pregabalin and Nortriptyline in their pharmaceutical preparations is simple, rapid and economic with less run time. The method has been validated and it has been shown that it is reliable, linear, accurate and precise as well as robust with minor variations in chromatographic parameters. Therefore, it can be applied for both routine analytical and quality control assay and it could be a very powerful tool to investigate the stability of Pregabalin and Nortriptyline.

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