DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR NORTRIPTYLINE AND PREGABALINE IN PHARMACEUTICAL FORMULATIONS

P.Hema Varshini
III B.Sc MPC, Ch.S.D.St.Theresa's College for Women(A), Eluru, Andhra Pradesh, India

S.Durgambica
III B.Sc MPC, Ch.S.D.St.Theresa's College for Women(A), Eluru, Andhra Pradesh, India

G.Srinivasa Rao
Department of Chemistry, Ch.S.D.St.Theresa's College for Women(A), Eluru, Andhra Pradesh, India

V.Naga Lakshmi
Department of Chemistry, Ch.S.D.St.Theresa's College for Women(A), Eluru, Andhra Pradesh, India

Received: Jul. 2019 Accepted: Aug. 2019 Published: Sep. 2019

Abstract: Simple, rapid, accurate and stability indicating High Performance Liquid Chromatographic method was developed and validated for the simultaneous determination of nortriptyline (NOR) and pregabaline (PRE) in pure and in pharmaceutical formulations using C_{18} Column and methanol: phosphate buffer in the ratio 75:25 v/v as mobile phase at pH 6.2 and flow rate of 0.9 mL/min with isocratic elution. The eluted compounds were detected using UV7000 detector at a wavelength of 234nm. The retention times of NOR and PRE were found to be 4.60 and 3.38 min, respectively, with a correlation coefficient of 0.9990 each. The linearity ranges for NOR and PRE were found to be 1-6µg/ml and 7.5-45µg/ml respectively. The LOD and LOQ values were observed to be 0.01µg/mL and 0.04µg/mL for NOR and 0.03µg/mL and 0.10µg/mL for PRE. The percentage recovery was found to be in range of 98.702 – 101.974 % and 99.092 –101.065 % for NOR and PRE, respectively. Both the drugs were subjected to acid, base, oxidation, photolytic and thermal degradation conditions. The degradation products of NOR and PRE were well resolved from the pure drug with significant differences in their retention time values. This validated method was applied for the simultaneous estimation of NOR and PRE in a commercially available formulation sample.

Keywords: Nortriptyline, Pregabaline, RP HPLC, Method Development and Validation.

Introduction: Pregabalin (PRE) comes under the class of anticonvulsant in medical terminology. It decreases the number of pain signals that are sent by damaged nerves in the human body thereby relieving the pain . It is chemically (S)-3-(amino methyl)-5-methylhexanoic acid

Nortriptyline Hydrochloride (NH) is an antidepressant drug and chemically it is 3-(10, 11-dihydro-5H-dibenzo, [a, d] cyclohept-5-ylidene) propyl (methyl) amine hydrochloride (MolWt-299.842g/mol). It inhibits the reuptake of the neurotransmitter serotonin at the neuronal membrane or acts at beta-adrenergic receptors it is used as an Antidepressant [1].
From the literature review, it is found that only a few analytical methods UV, HPLC, GC, and TLC are available for the estimation of NH in the combination with the other drugs [6-10] butthere is no any official stability indicating RP-HPLC method available till date for the estimation of Nortriptyline Hydrochloride in tablet dosage form [2-5].

Experimental: **Chemicals and Materials:** The analytical quality samples of NOR (99.88%) and PRE (99.68%) were received from Spectrum Pharmacy, Hyderabad, India as gift samples. The pharmaceutical formulation was procured from local market. Methanol and Milli-Q water used were HPLC grade and were purchased from Merck Specialties Private Limited, Mumbai, India. Buffer solutions used were AR Grade and purchased from Merck Specialties Private Limited, Mumbai, India.
Preparation of Mobile Phase: The mobile phase was prepared by mixing methanol: acetonitrile in the ratio of 50:40 (v/v) ratio and 1% sodium perchlorate 10 % (v/v) was added to adjust the pH 4.8. Mobile phase was sonicated for 15min and before use the mobile phase was filtered through 0.22µm membrane filter.

Preparation of Standard Solutions: 10mg of standard drug Nortriptyline and Pregabalin was accurately weighed separately and dissolved in 5ml diluent then transferred to a 10ml volumetric flask sonicate it for 5min, finally volume was made up to the mark with same solvent to make 1000µg/ml stock solution. From this 1ml was again diluted to 10ml to get a concentration of 100µg/ml solution of Nortriptyline and Pregabalin were obtained separately. From the solution, required concentration were prepared separately, then 1ml from each of the solution was mixed to obtained a combined solution for the simultaneous estimation of Nortriptyline and Pregabalin.

Results and Discussion: **Method Development:** Different trials are performed by taking different compositions of the mobile phases, but the peaks obtained are not clear and are unsymmetrical. Trials are also performed by taking different mobile phases like methanol and water, acetonitrile and water and also tried various columns like Kromosil 250, BDS 250, Symmetry C18 and Kromosil 8 but could not get satisfactory peaks. Different extraction methods were optimized for extraction of NOR and PRE from the tablet matrix and good recovery was obtained using methanol: phosphate buffer, in the composition ratio of 75:25. A good separation and elution were achieved methanol and phosphate buffer in the ratio of 75:25

was used as the mobile phase with a flow rate of 0.9 ml/min. Kromasil C-18 (250mm x 4.6mm, 5µm) column at 232 nm of UV detection.The validation parameters for the proposed analytical method are elucidated as per the guidelines of ICH (14). The achieved validation parameters are encapsulated in Table 1

Table I: Chromatography Conditions

S No	Parameter	Results
1	MP	Methanol: pH 6.2 Phosphate buffer 75:25 (v/v)
2	Wavelength	232nm
3	Stationary Phase	Kromasil C-18 (250mm x 4.6mm, 5µm) column
4	pH of MP	6.2
5	Flow Rate	0.9ml/min
6	Pump Mode	Isocratic
7	Pump Pressure	11.5±5MPa

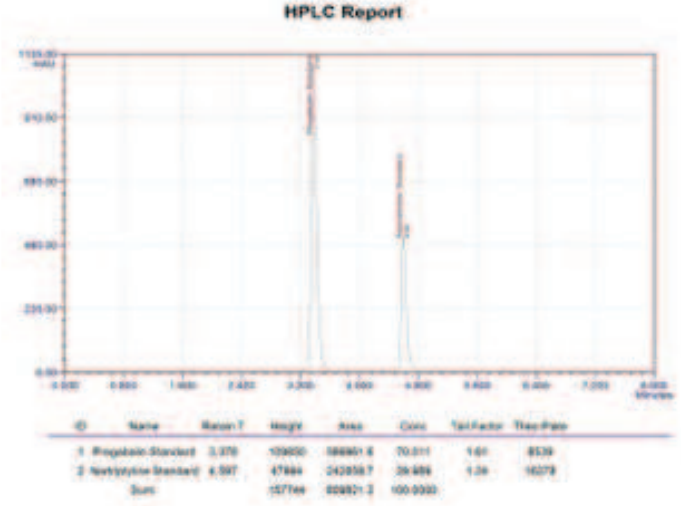

Fig. 2: Standard Chromatogram of NOR and PRE

System Suitability: The system suitability was evaluated by calculating the %RSD values of peak area, retention time, asymmetry and theoretical plates of five standard replicates. The experimental results (Table II) show that the values were within the acceptable range indicating that the system was suitable for the intended analysis.

Table II: System Suitability Test Results

S No	Parameter	Results
1	Api Concentration	Nortriptyline – 4µg/ml,Pregabalin - 30µg/ml
2	RT	Nortriptyline – 4.60min,Pregabalin - 3.38min
3	Resolution	Nortriptyline – 8.25,Pregabalin – ---
4	Area	Nortriptyline – 242859,Pregabalin - 566961
5	Theoretical Plates	Nortriptyline – 16378,Pregabalin - 8539
6	Tailing Factor	Nortriptyline – 1.24,Pregabalin e - 1.61

Specificity: In specificity study, standard solutions of PRE and NOR and the formulation placebo were injected and only drug peaks were obtained, which indicates that there was no interference from the excipients used and also from the mobile phase. The specificity study was also evaluated by examining the results of stress studies where the method is able to separate the main drug from the degradation products. Thus, specificity study ensures the selectivity of the developed analytical method, which is able to separate and quantify PRE and NOR in presence of different degradation products.

Linearity and Range: The linearity for the proposed HPLC method was established at six concentration levels by least squares linear regression method that represented no significant linearity deviation.

Table III: Linearity Results

Pregabalin		Nortriptyline	
Concentration	**Peak area**	**Concentration**	**Peak area**
7.5	156594	1	57835
15	290074	2	121621
22.5	426264	3	178139
30	566961	4	242859
37.5	712446	5	309144
45	874978	6	368977

Table IV: Accuracy Studies

Nortriptyline					
S.No	**Target Conc., (µg/ml)**	**Spiked conc., (µg/ml)**	**TotalConc., (µg/ml)**	**Conc., Obtained**	**% Recovery**
1	2	1	3	2.992	99.734
2	2	2	4	4.065	101.639
3	2	3	5	4.941	98.833
Pregabalin					
1	15	7.5	22.5	22.470	99.868
2	15	15	30	30.288	100.959
3	15	22.5	37.5	37.525	100.067
Average of three determinations					

Table V: Forced Degradation Results

S No	**Condition**	**No of additional peaks observed**	**Nortriptyline**			**Pregabalin**		
			Peak Area	**% Degradation**	**% Stability**	**Peak Area**	**% Degradation**	**% Stability**
1	Acidic	3	239528	1.37158	92.13	553515	2.37159	97.42
2	Base	4	223957	7.78312	94.56	549330	3.10974	98.51
3	Peroxide	3	234098	3.60744	98.89	520555	8.18504	98.48
4	Thermal	1	236906	2.45122	97.56	548318	3.28823	93.98
5	UV	3	216471	10.8656	91.09	548184	3.31187	96.32
6	Light	3	235770	2.91898	93.42	550208	2.95488	95.44

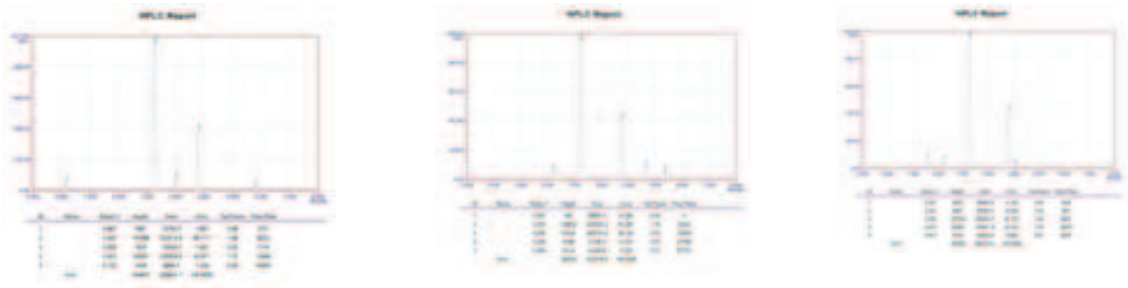

Fig.3,4,5: Acid, Alkali, Oxidative degradation studies chromatogram of NOR and PRE

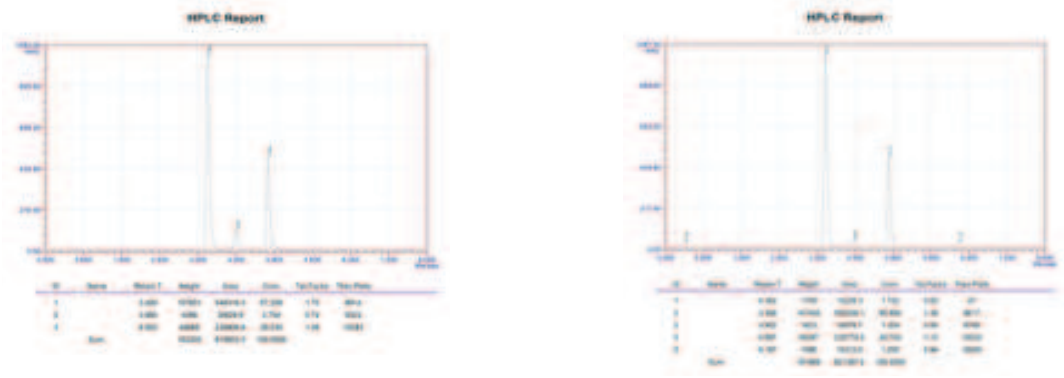

Fig.6,7: Thermal, Photodegradation Degradation Studies Chromatogram of NOR and PRE

Table VI: Results of Pharmaceutical Formulations

Drug	Brand Name	Available form	Label Claim	Amount Prepared	Amount Found	% Assay
Nortriptyline	Nortipan	Tablet	10mg	4µg/ml	3.957µg/ml	98.922
Pregabalin			75mg	30µg/ml	29.971µg/ml	99.904

Method Precision and Intermediate Precision: Precision studies were carried out by repeating the analysis of the sample six times and the results shows that the mean assay value and % RSD are found to be 0.300 and 0.491 for intraday precision and 1.309 and 0.401 for NOR and PRE, respectively, for inter day precision.

Accuracy: Accuracy of the method was studied by applying the developed method to prepared synthetic mixtures of formulation excipients to which known amount of NOR and PRE were added. Mean recovery (Table IV) for NOR was between 98.833–101.639% and 99.868–100.959% for PRE indicating that the developed method was accurate for the determination of NOR and PRE in pharmaceutical formulation.

LOD and LOQ: LOD value was found to be 0.01µg/mL and LOQ was 0.05µg/mL for NOR and 0.03µg/mL and 0.10µg/mL were the LOD & LOQ values for PRE, respectively.

Robustness: The robustness of the method was evaluated by assaying the same sample under different analytical conditions deliberately changed from the original analytical condition. The results obtained were not affected by varying the conditions and were in accordance with the results for original conditions. The change in %RSD value found to be 0.379-1.310 for NOR and 0.121-1.709% for PRE.

Solution Stability: The solution stability of the standard and the test sample solution was checked by analyzing both the solutions at interval of 12 h and 24 h at room temperature. The results showed that both the retention time and area of both the drugs were unchanged and no significant degradation was observed within the indicated period which was sufficient for performing analytical process.

Stress Degradation Study: The proposed validated liquid chromatographic method was successfully applied to study the stress degradation property of NOR and PRE. The results of forced degradation studies are given in Table V and Fig.4. Results indicate that the method has successfully separated the degradation products and identified separately. The results reveal that drugs were sensitive to the acidic conditions where more degradation occurred and stable to aqueous condition where no degradation was observed.

Conclusion: A rapid and efficient stability indicating RP–HPLC method has been developed for the simultaneous estimation of NOR and PRE in bulk and their combined dosage forms. The developed method was found to be accurate, precise, specific, sensitive, linear and robust on validation parameters. The validation results were found to be well within the limits. As the method separated the drug from its degraded products as well as degraded products each other, the method is stability indicating and can be conveniently used for the routine quality control analysis of NOR and PRE in industry for batch studies.

References:

1. Tripathi KD. Essentials of Medical Pharmacology, 6th Edn; Jaypee Brothers Publishers, New Delhi, 2003, pp 439-441.
2. European Pharmacopoeia; 6th Edn; European Pharmacopoeia Commission, Europe, 2015, pp 2120-2121.
3. Indian Pharmacopoeia. A government of India, Ministry of Health and Family Welfare, The Indian
4. Pharmacopoeia Commission, Ghaziabad, 2014, II, pp 1704-1705.
5. British Pharmacopoeia; 8th Edn; British Pharmacopoeia Commission, London, 2016, pp 775-777.
6. United State Pharmacopoeia and National Formulary, Asian Edn, United States Pharmacopoeia twin brook parkway, Rochville, MD, 2013, III, pp 4087-4088.

7. Anliker SL, Hamilton M, Bopp RJ and Goldberg MJ.Sensitive method for the quantitation of Nortriptyline and 10-hydroxy nortriptyline in human plasma by capillary gas chromatography with electron-capture detection.*J. Chrom*. **1992**, *573*, 141-145.
8. Zuleski FR, Loh A and Di Carlo FJ.Assay of human plasma for Nortriptyline by radioacetylation and Thinlayer Chromatography. *J. Chrom*. **1977**, *132*, 45-49.
9. Olesen OV, Plougmann P and Linnet K.Determination of Nortriptyline in human plasma by fully automatedsolid-phase extraction and online HPLC in the presence of antipsychotic drugs. *J. Chrom. B*. **2000**, *746*, 233-239.
10. Yazdi AS, Razavi N and Yazdinejad SR.Separation and determination of Amitriptyline and Nortriptyline by dispersive liquid-liquid microextraction combined with gas chromatography flame ionization detection.*Talanta*. **2008**, *75*, 1293-1299.
11. Faber DB, Mulder C, Man WA and Veld T.A thin layer method for the determination of Amitriptyline and Nortriptyline in plasma. *J. Chrom*. **1974**, *100*, 55-61.
12. Shen Y, Zhu RH, Deli H, Liu YW and Xu P.Validated LC-MS (ESI) assay for the simultaneous
13. determination of Amitriptyline and its metabolite Nortriptyline in rat plasma: Application to a pharmacokinetic comparison.*J. Pharm. Biomed. Ana*. **2010**, *53(3)*, 735-739.

***