DEVELOPMENT AND VALIDATION OF SPECTROPHOTOMETRIC METHODS FOR SIMULTANEOUS ESTIMATION OF NORTRIPTYLINE AND PREGABALIN IN PHARMACEUTICAL FORMULATIONS

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Abstract: *Objective:* To develop a simple, rapid, precise, accurate, sensitive spectrophotometric methods (A and B) were developed for simultaneous estimation and validation of Nortriptyline(NOR) and Pregabalin(PRE) in pure and tablet dosage forms.
Methods: Method A is a simultaneous equation method and Method B is a first-order derivative spectrophotometric method. Pure drug samples of NOR and PRE were dissolved in a ethanol and found to have absorbance maxima at 243.5 nm for NOR and 227nm for FFE, respectively.
Results: The linearity lies between 1-6 µg/ml for NOR and 7.5-45 µg/ml for PRE in these two methods (A and B). The correlation coefficient (r2) was found to be 0.999 and 0.998 for NOR and PRE, the limit of detection and limit of quantification were found to be 0.03 and 0.01 µg/ml for NOR and 0.15 and 0.5 µg/ml for PRE, respectively. The results of analysis have been validated statistically by recovery studies as per International Conference on Harmonization guidelines.
Conclusion: The two methods A and B showed good reproducibility and recovery with %RSD <2. Hence, both methods were found to be rapid, specific, precise, and accurate and can be

successfully applied for the routine analysis of NOR and PRE in pure and combined dosage form.

Keywords: Nortriptyline, Pregabalin, Derivative spectrophotometric, Simultaneous equation method, 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].

Equipment: Teccomp UV-2301 double beam UV-Visible spectrophotometer was used to carry out spectral analysis and the data was recorded by Hitachi software. Standard cuvettes of 10mm path length are used for analysis. Sonicator (1.3L) Ultrasonicator was used to sonicating the standard and formulation sample. Standard and sample drugs were weighed by using Denver electronic analytical balance (SI-234).

Preparation of Standard Drug Solution: 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

Method A: ***Simultaneous Equation Method:*** From the stock solution of 1000 µg/ml, working standard solutions of drugs were prepared by appropriate dilution and were scanned in entire UV range to determine the absorbance max. NOR has maximum absorbance at 243.5nm while PRE at 227nm (Fig. 2). Standard solutions were prepared having concentration 1-6 µg/ml for NOR and 7.5-45 µg/ml for PRE. At the absorbance's of these standard solutions, calibration curves were plotted at these wavelengths. Two simultaneous were formed using these absorptivity coefficient values.

$C_x = A_2a_{y1}-A_1a_{y2}/a_{x2}a_{y1}-a_{x1}a_{y2}, C_y = A_1a_{x2}-A_2a_{x1}/a_{x2}a_{y1}-a_{x1}a_{y2},$ Where, a_{x1} = Absorptivity of VTL at 231 nm, a_{x2} = Absorptivity of VTL at 260 nm, a_{y1} = Absorptivity of FFE at 260 nm, a_{y2} = Absorptivity of FFE at 231 nm

A1 and A2 are the absorbance of the diluted sample at 243.5 nm and 227 nm respectively.

Method B: ***First-Order Derivative Spectrophotometric Method:*** The absorption spectra thus obtained by working standard solutions of NOR and PRE in the wavelength range of 200-400 nm against solvent ethanol as blank were derivatized from first order. From the overlay spectra of both the drugs (Fig. 3), wavelengths selected for quantitation were 243.5nm was used for NOR and 227 nm was used for PRE. The proposed method was validated according to the United States Pharmacopeia and International Conference on Harmonization guidelines [49-51] in terms of linearity and range, precision, accuracy.

Results and Discussion:

Method A: ***Simultaneous Equation Method*****:** Study of overlain spectra shows that NOR has maximum absorbance at 243.5nm while FFE at 227 nm, respectively. The linearity with absorbance in the range 1-6 µg/ml for NOR and 7.5-45 µg/ml for PRE at their respective maxima was validated by least square method. Linearity results were presented in Table 1; calibration graphs were presented in Fig. 4. The accuracy of the method was determined by calculating mean percentage recovery. It was determined at 50,100 and 150% level. The percentage recovery ranges from98.66-99.733 for NOR and 99.013 to 99.833 for PRE, respectively. Precision was calculated as repeatability (%RSD <2) and inter and intraday variations (%RSD <2) for both drugs. The proposed methods were found to be simple, accurate and rapid for the routine determination of NOR and PRE in tablet formulation. Marketed brand of the tablet was analyzed, and the amount of drug determined by proposed methods was found to be 98.358-98.174 for NOR and PRE, respectively (Table 2). The method can be successfully used for simultaneous estimation of NOR and PRE in combined dosage form.

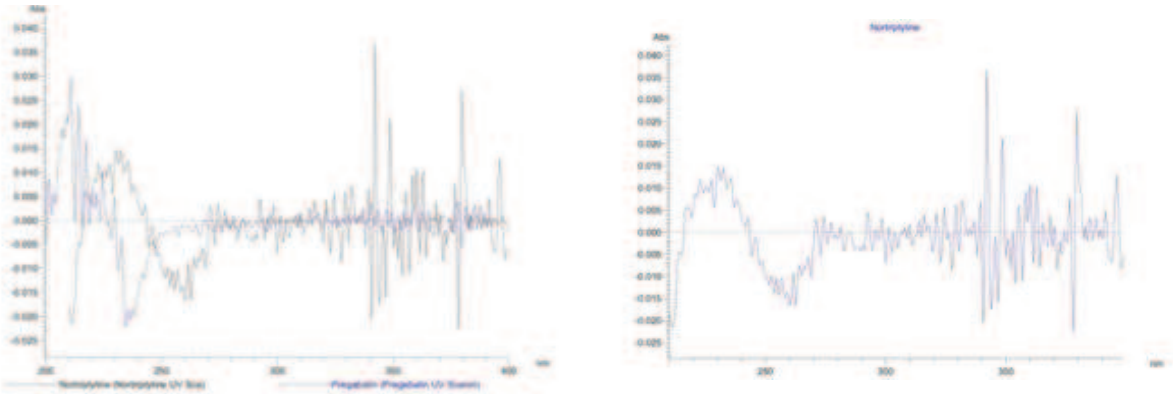

Fig.2,3,4: Overlay spectra of Nortriptyline and Pregabaline, Nortriptyline and Pregabaline

Method B: ***First-Order Derivative Spectrophotometric Method:*** Six points calibration curve were obtained in a concentration range from 1-6 µg/ml for NOR and 7.5-45 µg/ml for PRE, respectively. The response of the drug was found to be linear in the investigation concentration range and the linear regression equation was y = 0.003x - 0.003 with correlation coefficient (r2) 0.999 for NOR and y = 8E-05x + 0.002 with correlation coefficient (r2) 0.999 for PRE. The precision results were found to be within the limit where %RSD values for NOR found to be 0.512, 0.350, and 0.534 for intraday, interday and ruggedness studies. And also %RSD values for PRE found to be 0.163, 0.399 and 0.223 for intraday, interday, and ruggedness studies. Recovery results also found within the validation limit that percentage of recovery are 99.10-99.87 for NOR and 99.29-99.89 for PRE, respectively.

Table 1: Results of linearity

NOR		PRE	
Concentration	**Absorbance**	**Concentration**	**Absorbance**
1	0.663±0.006	7.5	0.209±0.015
2	0.661±0.008	15	0.368±0.023
3	0.662±0.007	22.5	0.492±0.045
4	0.663±0.003	30	0.663±0.056
5	0.661±0.009	37.5	0.795±0.078
6	0.661±0.006	45	0.968±0.011

The values given in table are the average±standard deviation for three replicate measurements of NOR: Nortriptyline, PRE: Pregabalin

Table 2: Formulation analysis of NOR and PRE by proposed methods

Method	Drug	Brand Name	Available form	Label Claim mg	Amount Prepared µg/ml	Amount Found µg/ml	% Assay
Simultaneous equation method	NOR	Nortipan	Tablet	10	4	3.934±0.0239	98.358
	PRE			75	30	29.452±0.0581	98.174
Derivative method	NOR	Nortipan	Tablet	10	4	3.951±0.016	98.775
	PRE			75	30	29.637±0.028	98.79

The values given in table are the average±standard deviation for three replicate measurements of NOR: Nortriptyline, PRE: Pregabalin

Analysis of Tabled Formulation: 3 formulation tablets from different batch numbers of Pregabalin and Nortriptyline [Nortipan; Pregabalin – 75mg and Nortriptyline – 10mg] were powdered. From the tablet powder, an amount equivalent to 10mg of Nortriptyline standard was weighed accurately and was dissolved in 10ml solvent. Sonicate the content for 10-15min to dissolve the drug completely in solvent. Then it was filtered and makes up to 10ml with same diluents to make 1000µg/ml Nortriptyline stock solution. Then it was diluted to get a sample concentration of 4µg/ml Nortriptyline sample solution. As per the label claim of the two drugs a Pregabalin concentration of 30µg/ml was obtained. The percentage recovery for NOR is 99.40 and PRE is 99.05, respectively The resultant solution was used for the simultaneous estimation of Pregabalin and Nortriptyline in combined dosage forms.

Conclusion: The proposed methods (A and B), i.e., simultaneous equation method and derivative method are found to be very simple and can be performed using any spectrophotometer and does not require much costly instruments. It also shows good linearity values and sensitivity. Thus, the methods are applicable for simple and economic estimation of NOR and PRE in their pharmaceutical dosage forms. Finally, it is also concluded that this was the first method developed in this combination of drugs NOR and PRE in their pharmaceutical dosage forms.

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