International Journal of Research in Pharmacy and Pharmaceutical Sciences
ISSN: 2455-698X; Impact Factor: RJIF 5.22
Received: 11-03-2019; Accepted: 13-04-2019
www.pharmacyjournal.in
Volume 4; Issue 3; May 2019; Page No. 72-77

Stability indicting liquid chromatography method for the simultaneous quantification of Nortriptyline and Pregabalin pharmaceutical formulations

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Abstract
A simple, accurate and stability indicating rapid High Performance Liquid Chromatographic method was developed and validated for the simultaneous determination of Nortriptyline and Pregabalin in pure and its pharmaceutical formulations using Hypersil ODS C18 column (250 X 4.6 mm, 5µ) as stationary phase and Acetonitrile, Methanol and 0.1M sodium perchlorate in the ratio of 40:30:30 (v/v) as mobile phase at pH 5.6, flow rate of 1 ml/min with isocratic elution. The eluted compounds were detected by using UV7000 detector at detection wavelength 205 nm. The retention times of Nortriptyline and Pregabalin are found to be 5.21 and 6.66 min respectively. The linearity ranges was 1-6 µg/ml and 7.5-45 µg/ml with LOD values 0.03 µg and 0.25 µg and LOQ values are 0.10 µg and 0.9 µg for Nortriptyline and Pregabalin respectively. Which were linear enough with correlation coefficient 0.999 in all the cases. The percentage recovery was found to be in range of 98.12 – 99.82% and 98.01 – 99.35% for Nortriptyline and Pregabalin respectively. The both the drugs were subjected to acid, base, hydrolysis, oxidation, photolytic and thermal degradation conditions. The degradation products of Nortriptyline and Pregabalin 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 Nortriptyline and Pregabalin in commercially available formulation sample.

Keywords: nortriptyline, pregabalin, RP HPLC, method development, validation

1. Introduction
Nortriptyline is a tricyclic antidepressant used in the treatment of depression. It is also used for chronic pain, anxiety disorders, bedwetting in children, attention-deficit/hyperactivity disorder (ADHD); and as an adjunctive therapy for smoking cessation [1]. Nortriptyline works by inhibiting the reuptake of serotonin and norepinephrine by the presynaptic neuronal membrane, thereby increasing the concentration of those neurotransmitters in the synapse [2]. The most serious adverse effects associated with the use of Nortriptyline includes include orthostatic hypotension, HTN, syncope, ventricular arrhythmia [3].
Pregabalin is an anticonvulsant drug used for neuropathic pain, epilepsy and generalized anxiety disorder [4]. Its use in epilepsy is as an add-on therapy for partial seizures [5]. Pregabalin relieves neuropathic pain (pain from damaged nerves) that can occur in your arms, hands, fingers, legs, feet, or toes. Exposure to pregabalin is associated with weight gain, sleepiness and fatigue, dizziness, leg swelling, disturbed vision, loss of coordination, and euphoria [6].

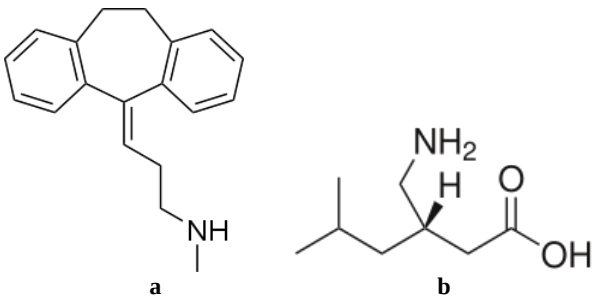

Fig 1: Chemical structures of Nortriptyline and Pregabalinin

Literature review for the analysis of Nortriptyline and Pregabalinin in pharmaceutical formulations confirms that only two HPLC methods were reported for the simultaneous analysis of these two drugs in pharmaceutical formulations [7, 8]. The pther methods reported were found to be estimation of Nortriptyline and Pregabalinin in singe or in combined with other simulkar action drugs [9-20]. Hence this paper describes a simple stability indicating HPLC method for the simultaneous quantification of Nortriptyline and Pregabalinin in pharmaceutical formulations.

2. Materials and Methods
2.1 Chemicals and Materials
Analytically pure Nortriptyline and Pregabalin were obtained as gift sample from reputed Pharmaceutical companies. Methanol, acetonitrile, water (Merck, Mumbai, India) was of HPLCgrade, while sodium perchlorate used for the preparation of mobile phase was of analytical grade (Merck Specialties Private Limited, Mumbai, India). The membrane filters 0.22 µm and syringe filters 0.45 µm for the analysis was supplied by Millpores (Millipores Ltd. Banglore). A formulation of PREKEM-NT having 75g of Pregabalin and 10mg of Nortriptyline was used for formulation analysis was procured from local Pharmacy.

2.2 Equipment
Agilent 1100 series HPLC with Quaternary G1311 A pump, COLCOM G1316A thermostat column temperature control, Thermostatic auto sampler G 1329A with sample volume of 0. 1 – 1500 µL and variable programmable UV detector G 1314 A. The instrument was operated and integrated with Agilent chem. station LC software.

2.3 Preparation of mobile phase
The mobile phase was prepared by mixing Acetonitrile, Methanol and 0.1M sodium perchlorate in the ratio of 40:30:30 (v/v) ratio and 1% sodium perchlorate 10 %(v/v) was added to adjust the pH at 5.6. Mobile phase was sonicated for 15min and before use the mobile phase was filtered through0.22 µm membrane filter.

2.4 Preparation of standard solutions
A stock solution of Nortriptyline and Pregabalin was prepared by dissolving 100 mg of the drug in 100 mL volumetric flask with methanol individually. Aliquots of this solution were suitability diluted with mobile phase to get working standard solutions of Nortriptyline and Pregabalin in the calibration concentration range.

2.5 Preparation of sample solution for assay
10 inhalation formulation blisters from 5 strips of Nortriptyline and Pregabalin (PREKEM-NT having 75g of Pregabalin and 10mg of Nortriptyline) were soaked in 5ml diluents and were keep it for solubility for 24H. Then it was filtered and makes up to 10ml with same diluents to make 100µg/ml stock solution. From this by proper dilution a concentration of 1µg/ml of Nortriptyline was prepared. As per the label claim of the two drugs a Pregabalin concentration of 7.50µg/ml was obtained. The resultant solution was used for the simultaneous estimation of Nortriptyline and Pregabalin in combined dosage forms.

2.6 Forced degradation studies
The stress degradation behaviour of the standard drugs Nortriptyline and Pregabalin in the developed method was studied in different stress degradation conditions like Acidic, Base, aqueous, Light, Peroxide, Thermal and UV Light conditions. In acidic, base and peroxide conditions, the standard 50 mg of each drug was mixed with 0.1 N HCl, 0.1 N NaOH and 3 % peroxide solution respectively. Then the drug was neutralized and diluted up to the standard concentration and were analyzed in the developed method. In Thermal and UV Light conditions the standard drug was incubated at 80ºC for thermal and under UV light for 24hr. Then the standard drugs were diluted and were analyzed in the developed method. The % degradation and the number of degradation products observed were calculated. The results of the forced degradation study confirm the stability of the developed method for Nortriptyline and Pregabalin.

3. Results and Discussion
3.1 Method development
After optimizing the several conditions for determination of Nortriptyline and Pregabalin mobile phase consisting of Acetonitrile, Methanol and 0.1M sodium perchlorate in the ratio of 40:30:30 (v/v) at pH 5.6 was found to be satisfactory. The drugs gave symmetric and sharp peaks with Hypersil ODS C18 column (250 X 4.6 mm, 5µ) column at 5.21min for Nortriptyline and 6.66 min for Pregabalin with good resolution, theoretical plates and acceptable tailing factor (figure 2). Wavelength was set at 205 nm, which provided better reproducibility with minimum interference.

Table 1: Chromatography conditions

Parameter	Results
MP	Acetonitrile, Methanol and 0.1M sodium perchlorate in the ratio of 40:30:30 (v/v)
Wavelength	205nm
Stationary Phase	Hypersil ODS C18 column (250 X 4.6 mm, 5µ)
pH of Mobile phase	5.6 with 1% Perchloric acid
Retention time Nortriptyline Pregabalin	 5.21 min 6.66 min
Flow Rate	1.0ml/min
Pump Mode	Isocratic
Pump Pressure	11.7±5MPa

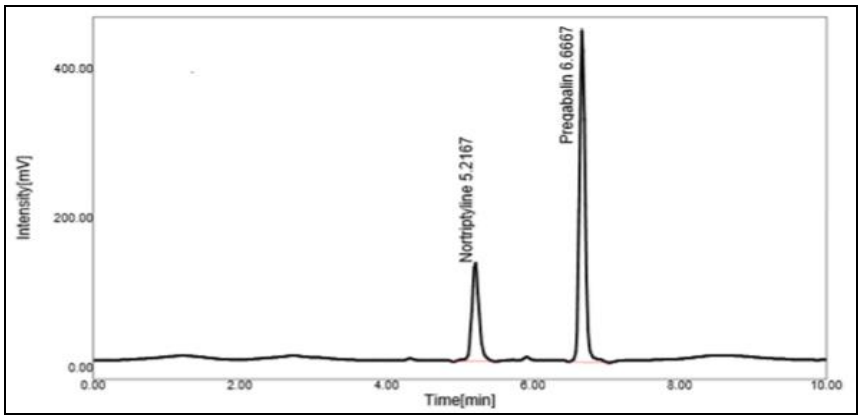

Fig 2: Standard chromatogram of Nortriptyline and Pregabalin

3.2 System suitability
The system suitability was evaluated by calculating the %RSD valuesof peak area, retention time, asymmetry and theoretical plates offive standard replicates. The experimental results (Table 2) showedthat the values were within the acceptable range indicating that thesystem was suitable for the intended analysis.

Table 2: System suitability test results

Parameter	Results
Api Concentration	Nortriptyline – 22.5µg/ml Pregabalin - 3µg/ml
RT	Nortriptyline – 5.21min Pregabalin – 6.66min
Resolution	Nortriptyline – ……. Pregabalin – 7.00
Area	Nortriptyline – 125248.5 Pregabalin – 310816.3
Theoretical Plates	Nortriptyline – 3148 Pregabalin - 7800
Tailing Factor	Nortriptyline – 0.52 Pregabalin – 0.91

3.3 Specificity

In specificity study, standard solutions of Nortriptyline and Pregabalin and the inhalation formulation placebo were injected and only drug peaks was obtained, whichindicates that there was no interference from the excipients usedand also from the mobile phase. The specificity study was alsoevaluated by examining the results of stress studies where themethod is able to separate the main drug from the degradationproducts. Thus specificity study ensures theselectivity of the developed analytical method which is able to separate and quantify Nortriptyline and Pregabalin in presence of different degradationproducts.

3.4 Linearity and range

The linearity of the developed method was determined at different concentrations ranging from 1- 6µg/ml for Nortriptyline and7.5-45µg/ml for Pregabalin. The regression analysis equation was y = 39835x + 4957. And correlation coefficient (r) was 0.999 for Nortriptyline and y = 13490x + 3649 and correlation coefficient (r) was 0.999 for Pregabalin respectively, showing good linearity. Theresults confirmed the linearity of the standard curves over the rangestudied and the excellent reproducibility of the assay method. Results and graphs of linearity study were reperesented in table 3 and figure 3 and 4.

Table 3: Linearity results

Nortriptyline		Pregabalin	
Concentration	**Peak area**	**Concentration**	**Peak area**
1	46352	7.5	102371
2	82341	15	207543
3	125248	22.5	310816
4	163274	30	403825
5	205176	37.5	513816
6	243893	45	608246

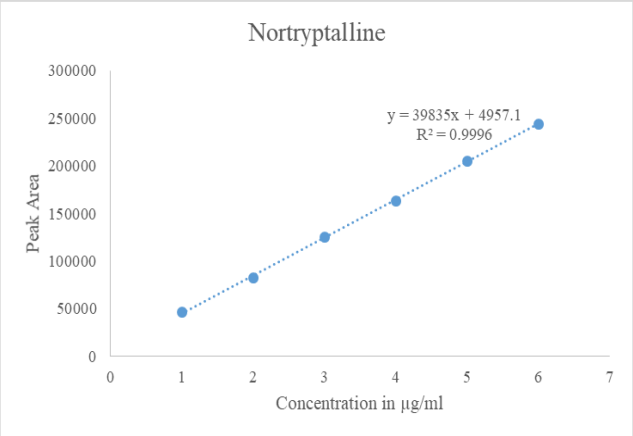

Fig 3: Calibration graph of linearity of Nortriptyline

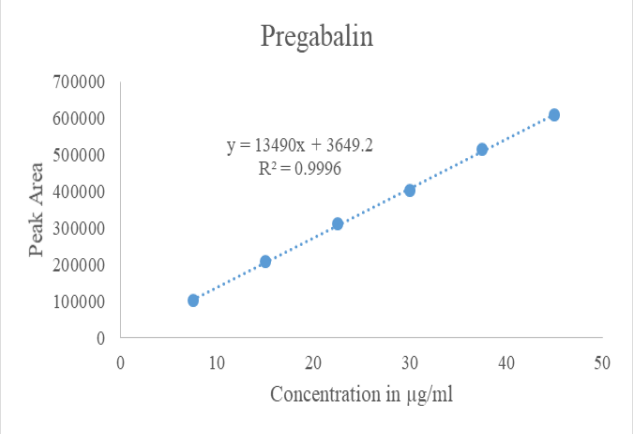

Fig 4: Calibration graph of linearity of Pregabalin

3.5 Method precision and intermediate precision

Precision studies were carried out by repeating the analysis of the samples six times and the results shows that the mean assay value and %RSD are found to be 1.49 and 0.67 for intraday precision and 1.37 and 0.68 for Nortriptyline and Pregabalin respectively.

3.6 Accuracy

Accuracy of the method was studied by applying the developedmethod to prepared synthetic mixtures of formulation excipients towhich known amount of Nortriptyline and Pregabalin. Mean recovery (Table 4 and 5) for Nortriptyline was between 98.12- 99.82% and 98.05-99.45 for Pregabalin indicating that the developed method was accurate for the determination of Nortriptyline and Pregabalin in pharmaceutical formulation.

Table 4: Results of Accuracy studies of Nortriptyline

S. No.	Level	Target	Spiked	Total	Peak Area	Amount found µg/ml	% Recovery
1	50%	2	1	3	122903.3	2.94384	98.12795
2		2	1	3	124839.8	2.99022	99.67409
3		2	1	3	123461.1	2.9572	98.57331
4	100%	2	2	4	160825.5	3.94001	98.50037
5		2	2	4	162980.7	3.99281	99.82036
6		2	2	4	161743.1	3.96249	99.06237
7	150%	2	3	5	203746.6	4.96517	99.30333
8		2	3	5	201812.9	4.91804	98.36087
9		2	3	5	202361.4	4.93141	98.6282

Table 5: Results of Accuracy studies of Pregabalin

S. No.	Level	Target	Spiked	Total	Peak Area	Amount found µg/ml	% Recovery
1	50%	15	7.5	22.5	304758.5	22.061497	98.0511
2		15	7.5	22.5	307461.1	22.2571385	98.9206
3		15	7.5	22.5	305283.3	22.0994873	98.2199
4	100%	15	15	30	399869.9	29.7061772	99.0206
5		15	15	30	397528	29.5321984	98.4407
6		15	15	30	396801.4	29.4782195	98.2607
7	150%	15	22.5	37.5	504937.9	36.8520467	98.2721
8		15	22.5	37.5	510476.5	37.2562722	99.3501
9		15	22.5	37.5	508753	37.1304854	99.0146

3.7 LOD and LOQ

LOD value was found to be 0.03µg/ml and LOQ was 0.10µg/ml for Nortriptyline and 0.25µg/ml and LOQ was 0.9µg/ml for Pregabalin respectively.

3.8 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 %assay value found to be 0.303-0.965 for Nortriptyline and 0.356-1.608 % for Pregabalin respectively.

3.9 Stress degradation study

The results of different stress degradation study of method developed for Nortriptyline and Pregabalin was given in table 6 and chromatograms was shown in Figure 5 to 9. The % degradation was found to be very high in Acidic degradation condition for both Nortriptyline and Pregabalin. In this condition four additional degradation products also observed and the % degradation was found to be 6.218 and 10.311 for Nortriptyline and Pregabalin respectively. In UV light degradation, a % degradation of 5.276 and 10.221 was observed for Nortriptyline and Pregabalin respectively with two additional degradation products. A very low % degradation of 2.667 and 3.616% observed in thermal and peroxide condition for Nortriptyline confirms that the drug was found to be more stable in these conditions. Pregabalin was found to be more stable in Peroxide conditions with a % degradation of 4.047%. In all the stress degradation conditions, the additional degradation products developed during the stress study were effectively separated and detected along with Nortriptyline and Pregabalin. Hence the method developed for the analysis of Nortriptyline and Pregabalin was stability indicating method.

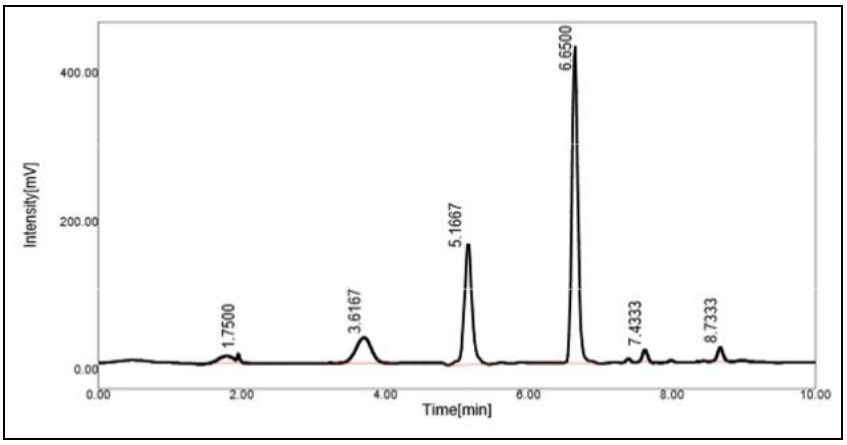

Fig 5: Forced degradation chromatograms of Nortriptyline and Pregabalin in acidic conditions

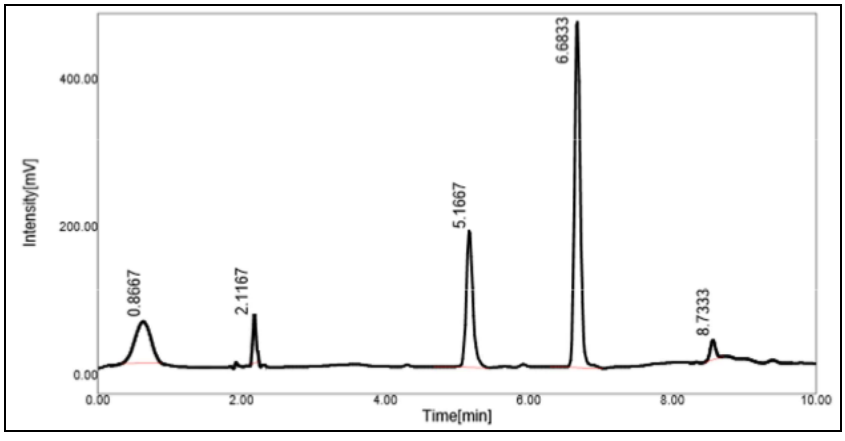

Fig 6: Forced degradation chromatograms of Nortriptyline and Pregabalin in base conditions

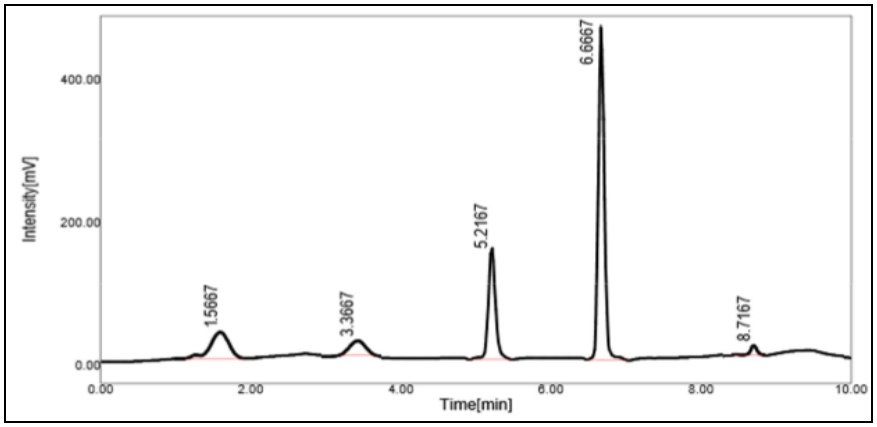

Fig 7: Forced degradation chromatograms of Nortriptyline and Pregabalin in peroxide conditions

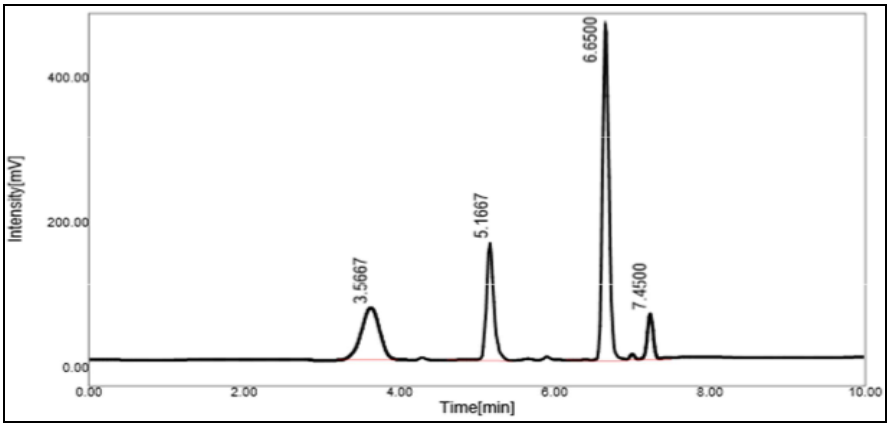

Fig 8: Forced degradation chromatograms of Nortriptyline and Pregabalin in thermal conditions

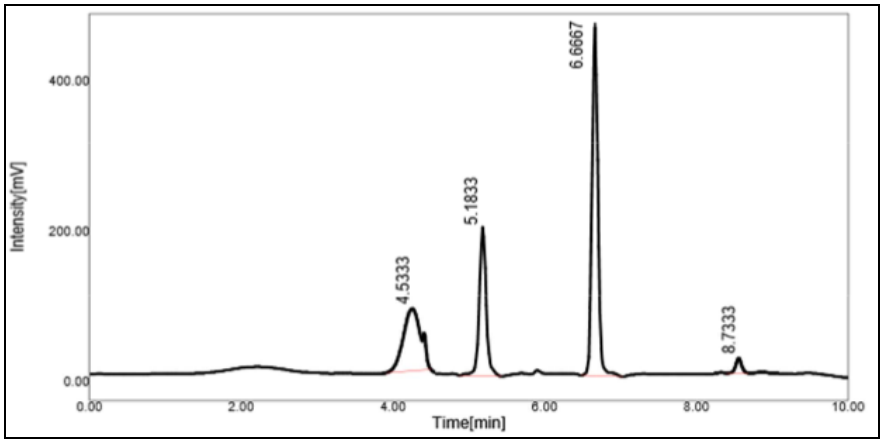

Fig 9: Forced degradation chromatograms of Nortriptyline and Pregabalin in UV conditions

Table 6: Forced Degradation results

Condition	Nortriptyline			Pregabalin		
	Area Obtained	**% Stability**	**% Degradation**	**Area Obtained**	**% Stability**	% Degradation
Acidic	117459.5	93.78	6.21	278769.1	89.69	10.31
base	119058.3	95.06	4.94	280139.4	90.13	9.86
Peroxide	120179.2	95.95	4.04	287963.3	92.64	7.35
Thermal	121894.6	97.32	2.67	299576.5	96.38	3.61
UV	118639.2	94.72	5.27	279047.9	89.77	10.22

Table 7: Results of Pharmaceutical formulations

S No	Brand name	Drug	Dosage	Concentration in µg/ml		% Assay
				Prepared	**Estimated**	
1	PREKEM-NT	Pregabalin	75mg	22.5	22.242	99.03
2		Nortriptyline	10g	3	2.971	98.85

4. Conclusion

A rapid and efficient RP–HPLC method has been developed for the simultaneous estimation and stability studies of Nortriptyline and Pregabalinin 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 with in the limit. As the method separated the drug from its depredated products as well as depredated products each other. The method is stability indicating, can be continently used for the routine quality control analysis of Nortriptyline and Pregabalinin industries for batch studies.

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