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Method Development and Validation for Simultaneous Estimation of Nor Triptyline And Pregabalin Pharmaceutical Dosage Forms Using Ultra Performance Liquid Chromatography (UPLC)

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ABSTRACT:

A straightforward and precise method was developed for the simultaneous quantification of Pregabalin and Nortriptyline in Tablet dosage form. The chromatogram was passed through an ACQUITY UPLC CSH C18 column with dimensions of 1.7 µm, 2.1 mm X 50 mm. The mobile phase, consisting of a buffer solution with a concentration of 0.01 OPA (ortho-phosphoric acid), mixed with acetonitrile in a ratio of 70:30, was passed through a column at a flow rate of 0.3 ml/min. The buffer utilized in this method was a 0.1% OPA buffer. The pH has been adjusted to 4.5. The temperature was consistently maintained at 30°C. The wavelength chosen for optimization was 225.0 nm. A straightforward and precise method was developed for the simultaneous estimation of Pregabalin and Nortriptyline in Tablet dosage form. The retention time of Pregabalin and Nortriptyline was determined to be 1.006 min and 1.339 min, respectively. The relative standard deviations (RSD) of Pregabalin and Nortriptyline were determined to be 0.3 and 0.5, respectively. The recovery rates for Pregabalin and Nortriptyline were 99.99% and 99.87% respectively. The limits of detection (LOD) and limits of quantification (LOQ) values derived from the regression equations for Pregabalin and Nortriptyline were 0.32 and 0.98 for Pregabalin, and 0.01 and 0.04 for Nortriptyline, respectively. The regression equation for Pregabalin is expressed as y = 31838x + 4526.2 y = 30657x + 97.143 of Nortriptyline. The quantity of Nortriptyline is 97.143 units. The method developed resulted in decreased retention times and run time, making it a simple and cost-effective option for regular quality control tests in industries.

Keywords: Nortriptyline, Pregabalin, UPLC, Method Validation, Stability Studies.

Introduction:

Neuropathic pain is a condition that arises following damage or disease to the somatosensory nervous system.[1] It is distinguished by an atypical heightened sensitivity to stimuli (excessive pain) and a sense of pain in response to non-painful stimuli (allergic).[2]The management of neuropathic pain primarily aims to address symptoms, and in certain pathological conditions, the underlying causes can be treated to alleviate pain,The Particular Interest Group on Neuropathic Pain recommended tricyclic antidepressants as the primary pharmacological treatment for neuropathic pain.[3]Combination therapy offers advantages over single-drug treatments by enhancing safety and efficacy, thereby facilitating the development of personalized therapies adapted to each patient. The combination of Pregabalin and Nortriptyline consists of two medications,The initial treatment options that are advised involve the administration of antidepressants and antiepileptic medications. Pregabalin acts as an alpha 2 delta ligand, reducing pain by regulating the calcium channel activity in nerve cells. Nortriptyline is a tricyclic antidepressant that enhances the concentrations of neurotransmitters (serotonin and noradrenaline) that inhibit the transmission of pain signals in the brain. Collectively, they alleviate neuropathic pain, which is pain resulting from nerve damage.[4,5]

Background:

Nortriptyline-Chemically known as $C_{19}H_{21}N$, Chemical Nomenclature of the drug given by IUPAC: - Methyl(tricyclo] pentadeca-1(15),3,5,7,11,13-hexaen-2-ylidene} propyl) amine.Nortriptyline is a tricyclic antidepressant prescribed for depression.[6] Is classified as a tricyclic antidepressant. It is employed for the management of severe depression and is additionally utilized off-label for chronic pain and other ailments.[7]This medication is prescribed to alleviate the symptoms of major depressive disorder. This drug can be used off-label to treat chronic pain, myofascial pain, neuralgia, and irritable bowel syndrome.[8] It work by inhibiting the serotonin and noradrenaline reuptake and reuptake of the neuronal membrane or acts at the level of the beta-adrenergic receptors.[9]

Pregabalin- Chemically known as $C_8H_{17}NO_2$, Chemical Nomenclature of the drug given by IUPAC-(3S)-3-(aminomethyl)-5-methylhexanoic acid,Pregabalin is an anticonvulsant medication employed for the management of neuropathic pain conditions and fibromyalgia, as well as for the treatment of partial onset seizures when used alongside other anticonvulsant drugs.[10]Pregabalin bears a structural resemblance to gamma-aminobutyric acid (GABA), which is an inhibitory neurotransmitter. It can be employed for the purpose of managing neuropathic pain, postherpetic neuralgia, and fibromyalgia, among other medical conditions,Although the structure of pregabalin is like gamma-aminobutyric acid (GABA), it does not bind to GABA

receptors, Instead, it binds the alpha2-delta subunit of presynaptic voltage-gated calcium channels in the central nervous system.[11,12]Nortriptyline, Pregabalin shown in (figure-1).

Nortriptyline **Pregabalin**

Figure-1: Structures of Nortriptyline, Pregabalin.

A comprehensive literature review revealed that numerous analytical methods have been documented, with the identification of more cost-effective approaches. However, no method has been reported for estimating stability studies. Therefore, a straightforward and economical method for determining the stability of Nortriptyline, Pregabalin, and in a pharmaceutical dosage form using UPLC is proposed.[13-21] must be developing and validated asper the guidelines of ICH (Q2 specification) [22].

Materials and Reagents.

Nortriptyline, Pregabalin, the respective pure drugs were acquired from Akrivis Pharma Pvt Ltd. The Nortriptyline, Pregabalin, combination tablet (Emaxgalin) was purchased from local market in Hyderabad. The chemicals and buffers utilized in this estimation were obtained from Rankem, an Indian supplier.

Instrumentation

The development and method validation were conducted using a WATERS HPLCACQUITY Premier System_Uplc, equipped with a TUv_detector. The system also included an automated sample injector and the Empower 2 software.

Objective:

The main aim of this study is to develop a highly dependable, precise, sensitive, specific, consistent, and efficient analytical technique for simultaneously measuring the amounts of Nortriptyline and Pregabalin in both their pure state and tablet form.

Chromatographic Conditions:

Flow rate: **0.3**ml/min

Column: CSH C18 Column,1.7 µm, 2.1 mm X 50mm

Buffer: 0.1% Ortho Phosphoric acid

Detector: 225.0 nm

Temperature: Ambient

Injection volume:5.0µL

Run time: 2.0 mins

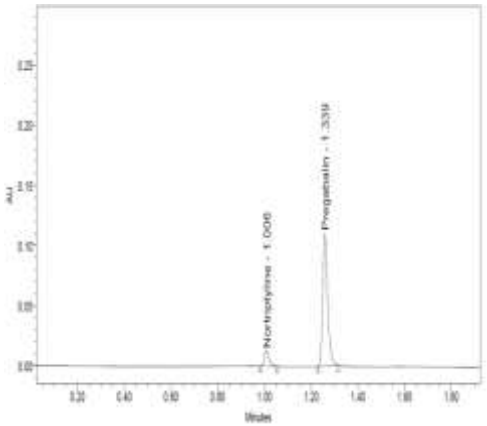

Fig 2 Optimized Chromatogram

Preparation of Buffer

Preparation of 0.1% Ortho Phosphoric acid:1ml of Conc Ortho Phosphoric acid was diluted to 1000ml with waterand the pH was adjusted to 5.4 using diluted Formic acid.

Preparation of Standard solution: Precisely measured 15 mg of Pregabalin and 2 mg of Nortriptyline were separately transferred to 50 ml volumetric flasks. Three-fourths of the diluents were added to both flasks, and then the mixture was sonicated for a duration of 10 minutes. The flasks were prepared by combining diluents and were then labeled as Standard stock solution 1 and 2. The concentration of Pregabalin is 300µg/ml and the concentration of Nortriptyline is 40µg/ml.

Preparation of Standard working solution: 1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (30µg/ml Pregabalin of and 4µg/ml of Nortriptyline)

Preparation of Sample solution: 10 tablets were weighed and the mean weight of each tablet was determined. The combination powder sample, which is equivalent to 75 mg and 10 mg of tablet, was precisely weighed and transferred into a 100ml volumetric flask. Then, 50ml of diluent was added and the mixture was subjected to sonication for 25 minutes. The volume was then adjusted by adding more diluent and the solution was filtered using milli-Q filters. The concentration of Pregabalin is 300µg/ml and the concentration of Nortriptyline is 40µg/ml.

Preparation of Sample working solution:1 ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent.(30µg/ml of Pregabalin and 4µg/ml of Nortriptyline)

Method Validation

The UPLC method was validated according to the ICH guidelines for the simultaneous estimation of the drug substances Nortriptyline and Pregabalin. This was conducted to demonstrate the method's suitability for regular analysis.

System suitability:

The system suitability parameters were established by creating standard solutions of Nortriptyline at a concentration of 4 parts per million (ppm) and Pregabalin at a concentration of 30 ppm. The solutions were subsequently administered six times in order to ascertain parameters such as peak tailing, resolution, and USP plate count. The RSD for the area of six standard injections must not surpass 2%. System suitability chromatogram was shown in figure 3 and values are mentioned in the table 1.

Specificity (Selectivity):

Verification of the interference in the optimized approach. No interfering peaks have been detected in the blank and placebo samples at the specific retention times of these drugs using this method. This method was described as specific. Figure 4 displays a representative chromatogram, while Table 2 provides the experimental data.

Table 1:System suitability results

S no	**Nortriptyline**			**Pregabalin**			
Inj	RT(min)	USP Plate Count	Tailing	RT (min)	USP Plate Count	Tailing	USP Resolution
1	1.002	3526	1.47	1.320	3362	1.58	2.7
2	1.003	3496	1.45	1.320	3162	1.54	2.8
3	1.003	3498	1.50	1.321	3082	1.51	2.7
4	1.003	3390	1.40	1.322	3015	1.53	2.7
5	1.005	3300	1.48	1.322	3014	1.46	2.7
6	1.006	3202	1.50	1.323	2715	1.45	2.6

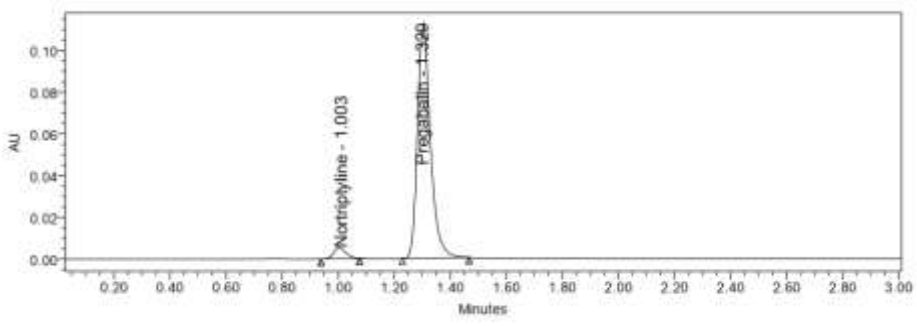

Figure 3: System suitability Chromatogram of Nortriptyline andPregabalin.

Table 2: Specificity data

Sample name	Retention time(mins)	Area
Nortriptyline	1.006	121053
Pregabalin	1.339	974636

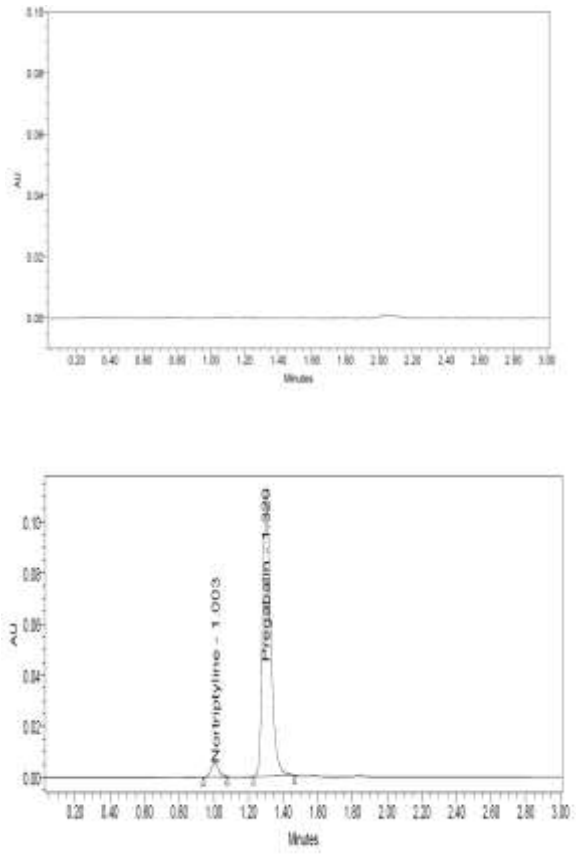

Figure 4: Specificity Chromatogram of Nortriptyline, and Pregabalin.

Table 3: Nortriptyline Linearity

% Level	CONC	Area
0	0	0
25%	1	28838
50%	2	61723
75%	3	94234
100%	4	124802
125%	5	151653
150%	6	183231
R^2 value		0.999

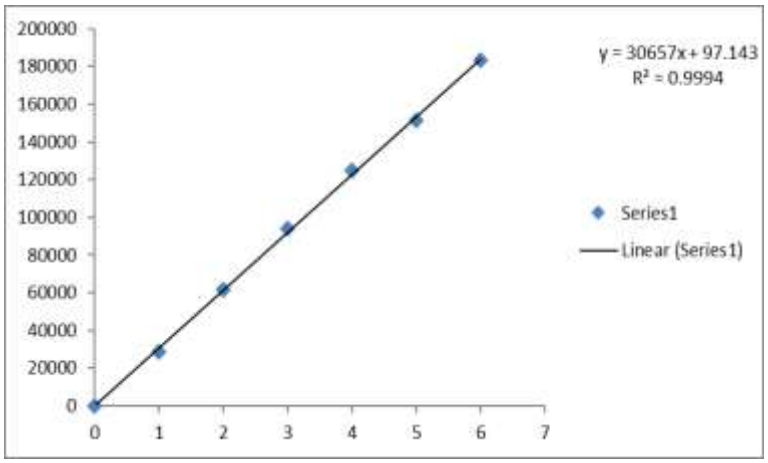

Figure 5: Nortriptyline Calibration curve

Table 4: Pregabalin Linearity

% Level	**CONC**	**Area**
0	0	0
25%	7.5	242539
50%	15	481289
75%	22.5	727159
100%	30	960631
125%	37.5	1212034
150%	45	1422575
R^2 value		0.999

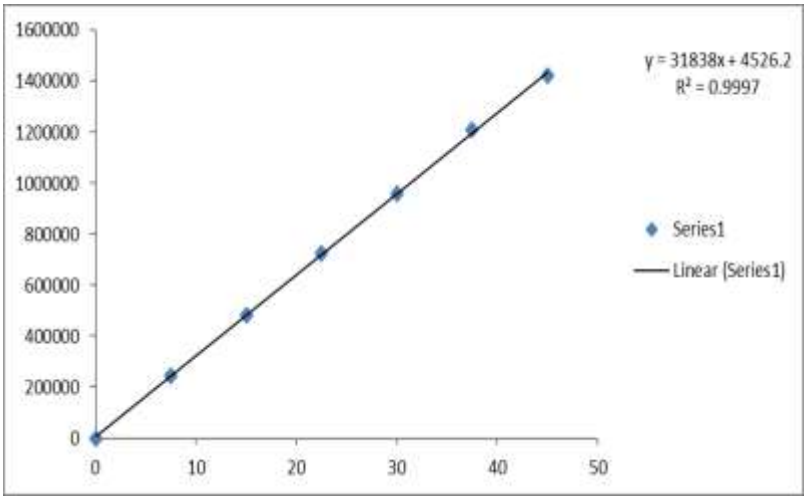

Figure 7: Pregabalin Calibration curve

Table 6:Accuracy (%Recovery data)

%Level	**Recovery Data**					
	Nortriptyline			**Pregabalin**		
	Amt added	Amt found	%Rec	Amt added	Amt found	%Rec
50% Level	2	2.01	100.58	15	15.03	100.20
	2	2.01	100.34	15	15.00	99.97
	2	1.98	99.14	15	14.96	99.76
100%Level	4	4.00	100.04	30	29.84	99.45
	4	4.03	100.68	30	30.12	100.41
	4	3.96	99.03	30	30.08	100.26
150%Level	6	5.99	99.85	45	44.68	99.28
	6	5.94	99.05	45	45.32	100.70
	6	6.01	100.13	45	44.94	99.87
Mean%			**99.87**			**99.99**

System Precision: The system precision was performed by analyzing six replicate injections of standard solution at 100% of the specified limit with respect to the working strength of Nortriptyline andPregabalin. Results of peak area are summarized in Table 6

Table 7: System precision data

Injection	**Nortriptyline**	**Pregabalin**
1	123526	979346
2	125248	978983
3	124053	978971
4	121053	965636
5	124402	974636
6	122325	975472
Avg	123416	975507
Std dev	1517.8	5234.8
%RSD	1.2	0.5

The % RSD for the peak areas of Nortriptyline and Pregabalin obtained from six replicate injections of standard solution was within the limit.

Method Precision: The precision of the method was determined by analyzing a sample of Nortriptyline andPregabalin. (Six individual sample preparations). Data obtained is summarized in Table 8.

Table 8: Method precision data

Injection	**Nortriptyline**	**Pregabalin**
1	123716	979112
2	122919	974585
3	123635	971850
4	124353	978767
5	124392	972568
6	123213	972142
Avg	123705	974837
Std dev	592.7	3320.2
%RSD	0.5	0.3

From the above results, the % RSD of method precision study was within the limit for Nortriptyline and Pregabalin.

Table 9: Robustness results: -The robustness conditions, including a flow rate decrease of 0.2ml/min, a flow rate increase of 0.4ml/min, a decrease in mobile phase composition to 65% B and 35% A, an increase in mobile phase composition to 75% B and 25% A, a decrease in temperature to 25°C, and an increase in temperature to 35°C, were maintained. The samples were injected in duplicate. The system suitability parameters were minimally impacted and all parameters met the required criteria. The %RSD value fell within the specified limit.

Chromatographic condition	**Pregabalin (RSD)**	**Nortriptyline (RSD)**
Flow rate (-) 0.2ml/min	0.2	0.4
Flow rate (+) 0.4ml/min	0.5	0.4
Mobile phase (-) 35:65A	0.7	0.5
Mobile phase (+) 25B:75A	3.4	0.9
Temperature (-) 25°C	0.2	1.0
Temperature (+) 35°C	0.3	1.0

Table 10: Forced degradation conditions for Nortriptyline andPregabalin.

Stress condition	**Solvent**	**Temp(^{0}C)**	**Exposed time**
Acid	2N HCL	60^0c	30 mins
Base	2N NAOH	60^0c	30 mins
Oxidation	20% H_2O_2	60^0c	30 mins
Thermal	Diluent	105^0c	6 hours
Photolytic	Diluent	-	-
Hydrolytic	Water	60^0c	

No degradation was detected in the samples when they were subjected to acid, base, hydrolysis, thermal, light, and water. Based on the stress study, none of the degradation products co-eluted with the peaks formed by the active drug.

Table 11: Degradation profile results

Type of degradation	**Pregabalin**			**Nortriptyline**		
	AREA	%RECOVERED	% DEGRADED	AREA	%RECOVERED	% DEGRADED
Acid	915458	93.75	6.25	116251	93.90	6.10
Base	949361	97.22	2.78	120511	97.34	2.66
Peroxide	943412	96.61	3.39	118810	95.96	4.04
Thermal	965802	98.91	1.09	120847	97.61	2.39
Uv	968893	99.22	0.78	121040	97.77	2.23
Water	969642	99.30	0.70	122752	99.15	0.85

Table 12: Assay results for Nortriptyline and Pregabalin

(Cabenuva), bearing the label claim Nortriptyline and Pregabalin10MG, 75MG. Assay were performed with the above formulation.

	Label claim dose	**%Assay**
Nortriptyline	10mg	99.93
Pregabalin	75mg	99.83

Assay was performed by: -

The weight of 10 tablets was measured and the average weight of each tablet was calculated. Subsequently, the weight attributed to a single tablet was transferred into a volumetric flask with a capacity of 100 ml. A total of 50 milliliters of diluent was added, and the mixture underwent sonication for a duration of 25 minutes. Afterwards, the volume was modified with a diluent and then passed through UPLC filters for filtration. The Nortriptyline concentration is 100µg/ml and the Pregabalin concentration is 750µg/ml. Preparation of the working solution sample: 1 milliliter of the filtered sample stock solution was transferred into a 10-milliliter volumetric flask and then filled with diluent to the top. The concentration of Nortriptyline is 4 µg/ml and the concentration of Pregabalin is 30 µg/ml. After injecting six samples of the formulation, the relative standard deviation (RSD) for the area of the six standard injections should not exceed 2%.

Assay was calculated by: -

$$\% \text{ Assay} = \frac{AT}{AS} \times \frac{WS}{50} \times \frac{1}{10} \times \frac{100}{1} \times \frac{10}{0.4} \times \frac{P}{100} \times \frac{FV}{L.C} \times 100$$

AT	Average Peak area of test solution
AS	Mean peak area of standard solution
WS	Weight of working standard taken in mg
P	Assay of working standard in % on dried basis
L.C	Label Claim
Avwt	Average weight of a drug

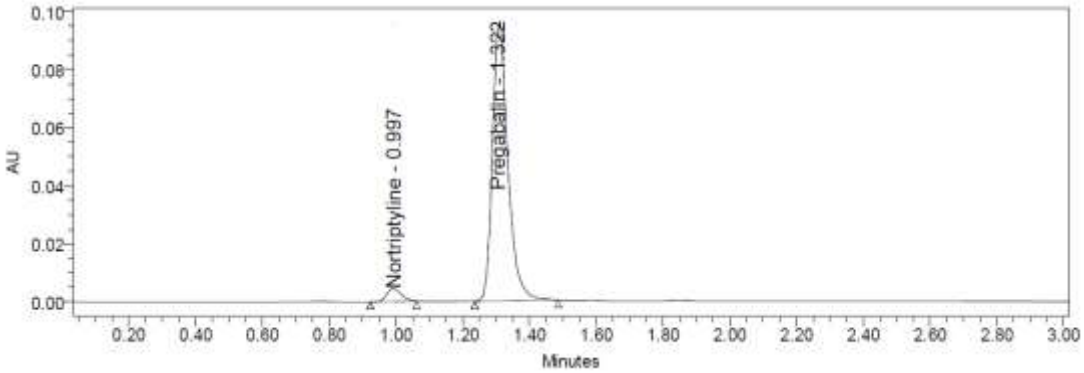

Figure 8: Assay Chromotogram

CONCLUSION

A novel and validated stability indicating analytical approach was developed using UPLC methodology. The study's results will significantly contribute to the monitoring of the quality of Nortriptyline and Pregabalin in pharmaceutical dosage forms. This is attributed to the study's uncomplicated sample preparation technique, which employs a minimal quantity of mobile phase and necessitates only a short analysis duration. After examining two medications in a combined dosage form, the results showed a nearly perfect effectiveness of 100% using the newly developed methodology. The recovery studies yielded positive results, suggesting that the excipient has no discernible impact. **ACKNOWLEDGEMENT:**

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