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Development and Validation of an HPLC Method for Simultaneous Determination of Nortriptyline and Pregabaline in Pharmaceutical Dosage Form

Karuru Anil*[1], R. Kiran Jyothi [2], M. Mahesh[3]
*[1]*Student, Department of Pharmaceutical analysis,Oil Technological & Pharmaceutical Research Institute, JNTU,Anantapur,Andhra Pradesh, India.*
2,3 Department of Pharmaceutical analysis, Oil Technological & Pharmaceutical Research Institute, JNTU, Anantapur, AndhraPradesh, India.

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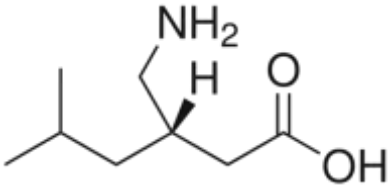
ABSTRACT: The assay of Pregabalin and Nortriptyline was performed with tablets and the % assay was found to be 99.58 and 99.61 which shows that the method is useful for routine analysis.
The linearity of Pregabalin and Nortriptyline was found to be linear with a correlation coefficient of 0.999 and 0.999, which shows that the method is capable of producing good sensitivity.
The acceptance criteria of precision is RSD should be not more than 2.0% and the method show precision 0.33 and 0.27 for Pregabalin and Nortriptyline which shows that the method is precise.
The acceptance criteria of intermediate precision is RSD should be not more than 2.0% and the method show precision 0.47 and 0.30 for Pregabalin and Nortriptyline which shows that the method is repeatable when performed in different days also.
The accuracy limit is the percentage recovery should be in the range of 97.0% - 103.0%. The total recovery was found to be 99.09% and 100.73% for Pregabalin and Nortriptyline. The validation of developed method shows that the accuracy is well within the limit, which shows that the method is capable of showing good accuracy and reproducibility.
KEYWORDS: Pregabalin, Nortriptyline, RSD, accuracy

I. INTRODUCTION

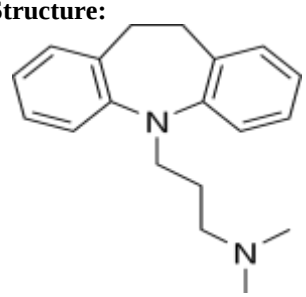
PREGABALIN:
Pregabalin is an anticonvulsant drug used to treat neuropathic pain conditions and fibromyalgia, and for the treatment of partial onset seizures in combination with other anticonvulsants.

Chemical structure:



IUPACNAME:(3S)-3-(aminomethyl)-5-methylhexanoic acid
Chemical Formula: $C_8H_{17}NO_2$
Molecular weight:159.22
Melting point:176 - 178ºC
Pka: 4.8

2. NORTRIPTYLINE:
Description: Nortriptyline is a tricyclic antidepressant used in the treatment of depression
Chemical Structure:



Formula : $C_{19}H_{21}N$
Molecular weight : 263.37
Freely soluble : Methanol

PHARMACOLOGY:
Pharmacodynamics:
Nortriptyline exerts antidepressant effects likely by inhibiting the reuptake of serotonin and norepinephrine at neuronal cell membranes. It also exerts antimuscarinic effects through its actions on the acetylcholine receptor**.**

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Mechanism of action:

Though prescribing information does not identify a specific mechanism of action for nortriptyline[18], is believed that nortriptyline either inhibits the reuptake of the neurotransmitter serotonin at the neuronal membrane or acts at the level of the beta-adrenergic receptors. It displays a more selective reuptake inhibition for noradrenaline, which may explain increased symptom improvement after nortriptyline therapy.[16] Tricyclic antidepressants do not inhibit monoamine oxidase nor do they affect dopamine reuptake.[18] As with other tricyclics, nortriptyline displays affinity for other receptors including mACh receptors, histamine receptors, 5-HT receptors, in addition to other receptors

Brand Names:

II. METHODOLOGY

HPLC METHOD DEVELOPMENT:

Wave length selection:

UV spectrum of 10 µg / ml Pregabalin and Nortriptyline in diluents (mobile phase composition) was recorded by scanning in the range of 200nm to 400nm. From the UV spectrum wavelength selected as 227. At this wavelength both the drugs show good absorbance.

Optimization of Column: Waters Symmetry C_{18} (4.6 x 250mm, 5.0µm) was found to be ideal as it gave good peak shape and resolution at 1.2 ml/min flow.

OPTIMIZEDCHROMATOGRAPHIC CONDITIONS:

Instrument used : High performance liquid chromatography equipped with Auto Sampler and PDA detector
Temperature:Ambient
Column : Waters Symmetry C_{18} (4.6 x 250mm, 5.0µm)
Buffer: 0.1% ortho phosphoric acid buffer
Mobile phase:25% buffer: 75% Methanol
Flow rate: 1.0 ml per min
Wavelength:227 nm
Injection volume : 20 µl
Run time: 10min.
Optimized chromatogram is shown in the **fig 2.** System suitability parameters are shown in **fig 2** and theresults are shown in **Table 6.**

PREPARATION OF BUFFER AND MOBILE PHASE:

Preparation of 0.1% Ortho phosphoric acid buffer:

Pipetted 1 ml of ortho phosphoric acid in 100 ml HPLC water.

Preparation of mobile phase:

Mix a mixture of above buffer 250 ml (25%) and 750 ml MethonolHPLC (75%) and degas in ultrasonic water bath for 5 minutes. Filter through 045 µ filter under vacuum filtration.

Diluent Preparation:

Use the Mobile phase as Diluents.

VALIDATION PARAMETERS:

1. ASSAY:

Standard Solution Preparation:

Accurately weigh and transfer 75mg of Pregabalin & 10mg of Nortriptyline working standard into a 100ml clean dry volumetric flask add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 1.5 ml of Pregabalin & Nortriptyline of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

Sample Solution Preparation:

Accurately weigh and transfer equivalent to 75mg of Pregabalin & 10mg Nortriptyline equivalent weight of the sample into a 100ml clean dry volumetric flask add about 70ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Further pipette 1.5 ml of Pregabalin & Nortriptyline of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

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Procedure: Inject 20 µL of the standard, sample into the chromatographic system and measure the areas for the Pregabalin & Nortriptyline peaks and calculate the %Assay by using the formulae. The chromatograms were recorded as show in **Fig 3, 4** andresults are shown in **Table- 7.**

2. LINEARITY:

Preparation of stock solution:

Accurately weigh and transfer 75mg of Pregabalin & 10mg of Nortriptyline working standard into a 100ml clean dry volumetric flask add about 70mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Preparation of Level – I (37.5 ppm & 5 ppm of Pregabalin & Nortriptyline):

0.5 ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with Diluents.

Preparation of Level – II (75 ppm & 10 ppm of Pregabalin & Nortriptyline):

1.0 ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with Diluents.

Preparation of Level – III (112.5 ppm & 15 ppm of Pregabalin & Nortriptyline):

1.5 ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with Diluents.

Preparation of Level – IV (150 ppm & 20ppm of Pregabalin & Nortriptyline):

2.0 ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with Diluents.

Preparation of Level – V (187.5 ppm & 25 ppm of Pregabalin & Nortriptyline):

5ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with Diluents.

Procedure: Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

The chromatograms were recorded as show in **Fig 5 – 9,** calibrationgraphs were shown in **Fig 10,11** andresults are shown in **Table-8, 9.**

3. PRECISION:

Preparation of stock Solution:

Accurately weigh and transfer 75mg of Pregabalin & 10mg of Nortriptyline working standard into a 100ml clean dry volumetric flask add about 70ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 1.5 ml of Pregabalin & Nortriptyline the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

Procedure: The standard solution was injected for six times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

The sample solutions of Pregabalin and Nortriptyline chromatograms were recorded as shown in **Fig 12- 16.** The mean and percentage relative standard deviation were calculated from the peak areas and shown in the **Table 10 and 11.**

4. Intermediate Precision/Ruggedness: To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different day within the laboratory.

Preparation of stock solution:

Accurately weigh and transfer 75mg of Pregabalin & 10mg of Nortriptyline working standard into a 100ml clean dry volumetric flask add about 70ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 1.5ml of Pregabalin & Nortriptyline the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

Procedure: The standard solution was injected for Six times and measured the area for all Five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Chromatograms were recorded as shown in **Fig 17-22** and results are shown in **Table 12 and 13.**

5. ACCURACY:

For accuracy determination, three different concentrations were prepared separately i.e. 50%, 100% and 150% for the analyte and chromatograms are recorded for the same.

Preparation of Standard stock solution:

Accurately weigh and transfer 75mg of Pregabalin & 10mg of Nortriptyline working standard into a 100ml clean dry volumetric flask add about 70ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 1.5 ml of Pregabalin &Nortriptyline the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

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Preparation Sample solutions:

For preparation of 50% solution (With respect to target Assay concentration):

Accurately weigh and transfer 37.5mg of Pregabalin and 5mg of Nortriptyline working standard into a 100ml clean dry volumetric flask add about 70 ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 1.5 ml of Pregabalin & Nortriptyline the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents

For preparation of 100% solution (With respect to target Assay concentration):

Accurately weigh and transfer 75mg of Pregabalin and 10mg of Nortriptylineworking standard into a into a 100ml clean dry volumetric flask add about 70ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).
Further pipette 1.5 ml of Pregabalin & Nortriptyline the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

For preparation of 150% solution (With respect to target Assay concentration):

Accurately weigh and transfer 112.5mg of Pregabalin and 15mg of Nortriptyline equivalent weight of tablet powder into a 100ml clean dry volumetric flask add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).Further pipette 1.5 ml of Pregabalin & Nortriptyline the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

TABLE 4: INSTRUMENTS USED

SL. No	Instrument	Model
1	HPLC	WATERS, software: Empower, 2695 separation module.2487 UV detector.
2	UV/VIS spectrophotometer	LABINDIA UV 3000^{+}
3	pH meter	Adwa – AD 1020
4	Weighing machine	Afcoset ER-200A
5	Pipettes and Burettes	Borosil
6	Beakers	Borosil

TABLE 5: CHEMICALS USED

SL.No	Chemical	Brand
1	Pregabalin	Supplied by Kenna Helath care
2	Nortriptyline	Supplied by Kenna Helath care
3	Ortho phosphoric acid	FINAR chemical LTD
4	Water and Methanol for HPLC	Standard solutions Ltd
5	Acetonitrile for HPLC	Standard solutions Ltd
6	HCl, H_2O_2, NaOH	MERCK

III. RESULTS AND DISCUSSION

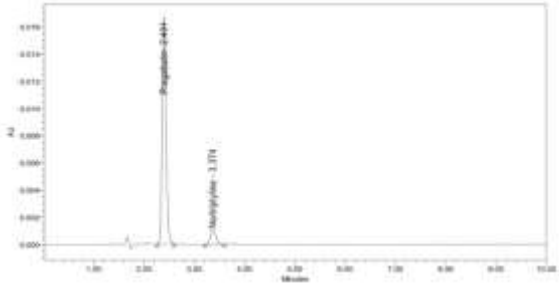

FIGURE 2: CHROMATOGRAM FOR SYSTEM SUITABILITY

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TABLE 6: RESULTS OF SYSTEM SUITABILITY PARAMETERS

S.No	Name	RT(min)	Area (µV sec)	Height (µV)	USP resolution	USP tailing	USP plate count
1	Pregabalin	2.401	2094603	196622		1.71	2947.68
2	Nortriptyline	3.374	3694090	286174	4.38	1.61	3826.77

Acceptance criteria:

- Resolution between two drugs must be not less than 2.
- Theoretical plates must be not less than 2000.
- Tailing factor must be not more than 2.
- It was found from above data that all the system suitability parameters for developed method were within the limit.

VALIDATION PARAMETERS:

1. ASSAY:

Standard and sample solution injected as described under experimental work. The corresponding chromatograms and results are shown below.

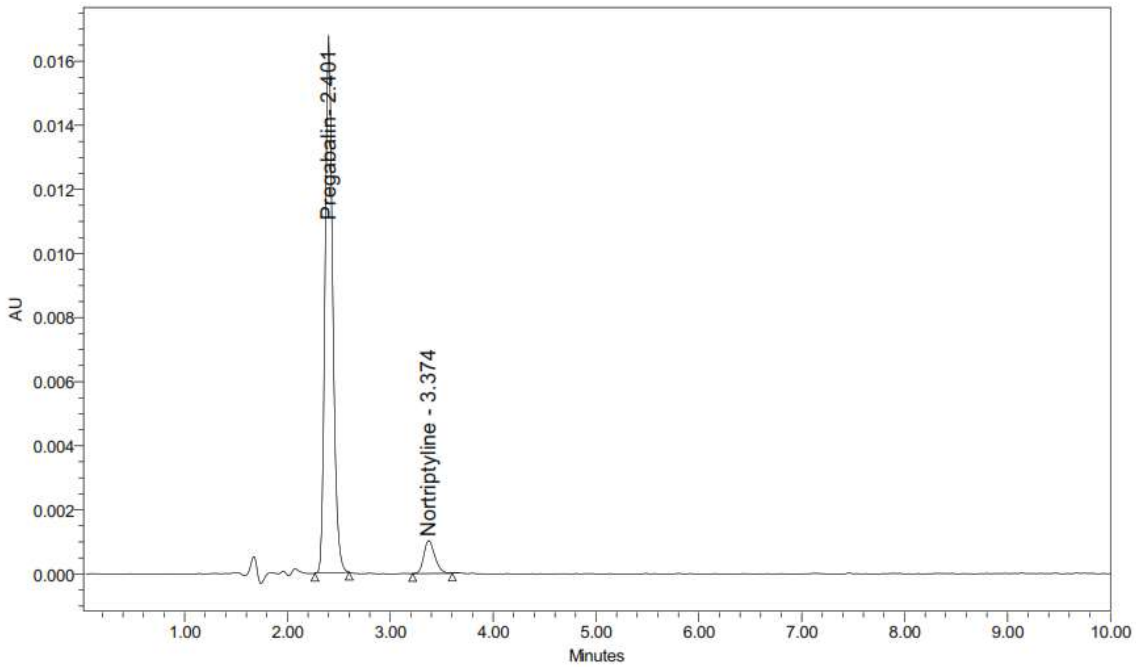

FIGURE 3: CHROMATOGRAM FOR STANDARD

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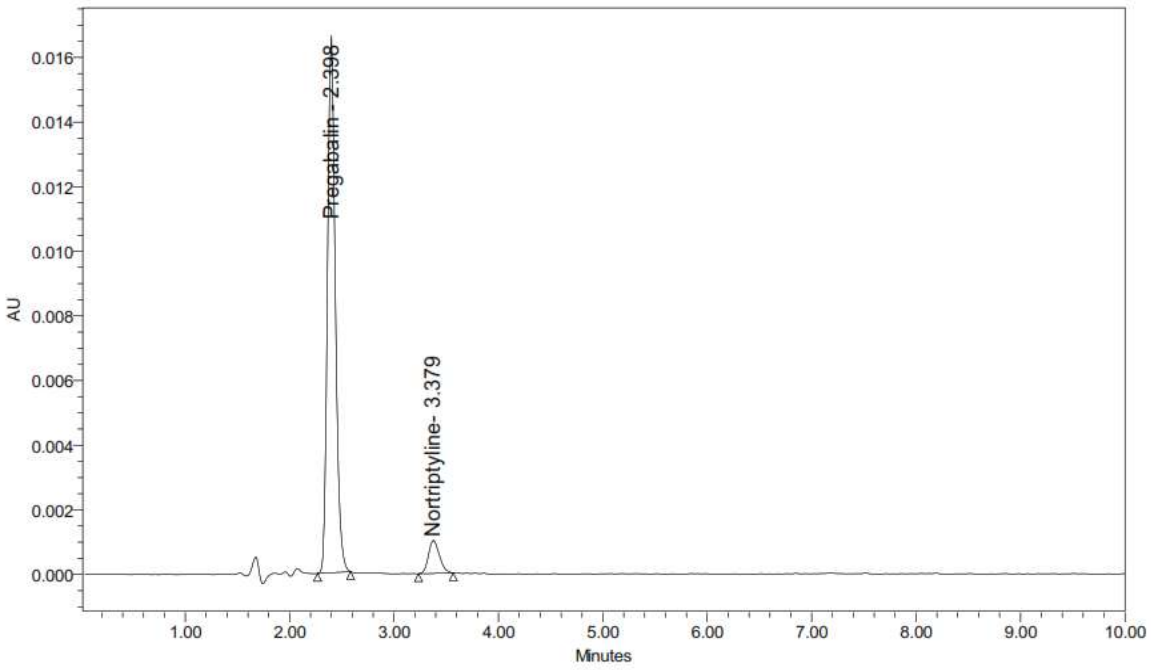

FIGURE 4: CHROMATOGRAM FOR SAMPLE

TABLE 7: RESULTS OF ASSAY FOR PREGABALIN AND NORTRIPTYLINE

	LABEL CLAIM (MG)	% ASSAY
PREGABALIN	75	99.58
NORTRIPTYLINE	10	99.61

2. LINEARITY:

THE LINEARITY RANGE WAS FOUND TO LIE FROM 1µG/ML TO 5µG/ML OF PREGABALIN, 7.5µG/ML TO 37.5µG/ML 0F NORTRIPTYLINE AND CHROMATOGRAMS ARE SHOWN BELOW.

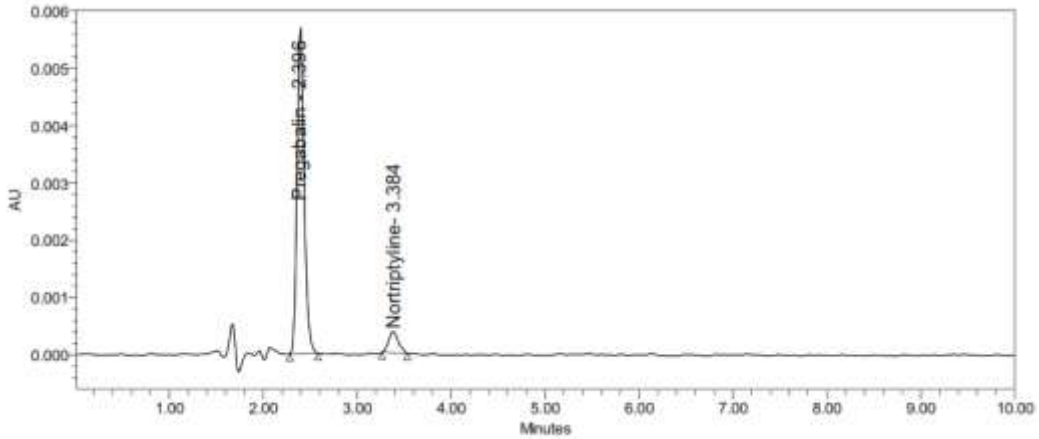

FIGURE 5: CHROMATOGRAM FOR LINEARITY-1

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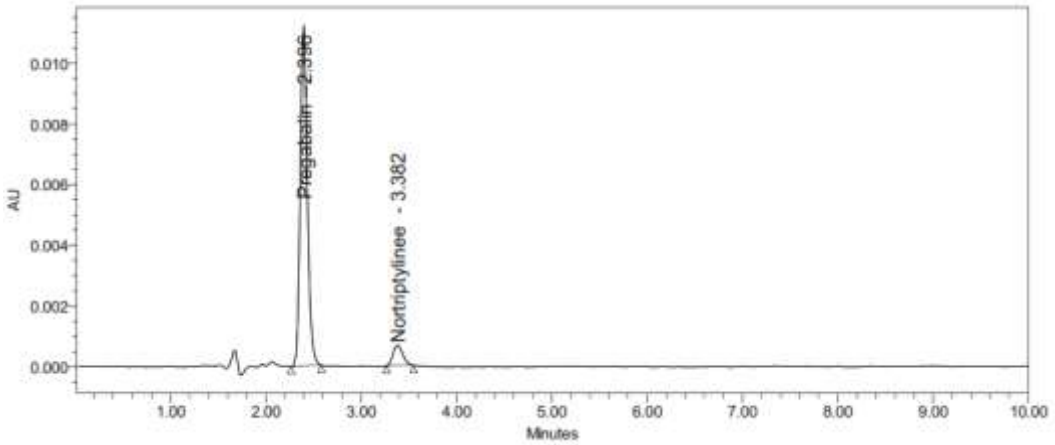

FIGURE 6: CHROMATOGRAM FOR LINEARITY-2

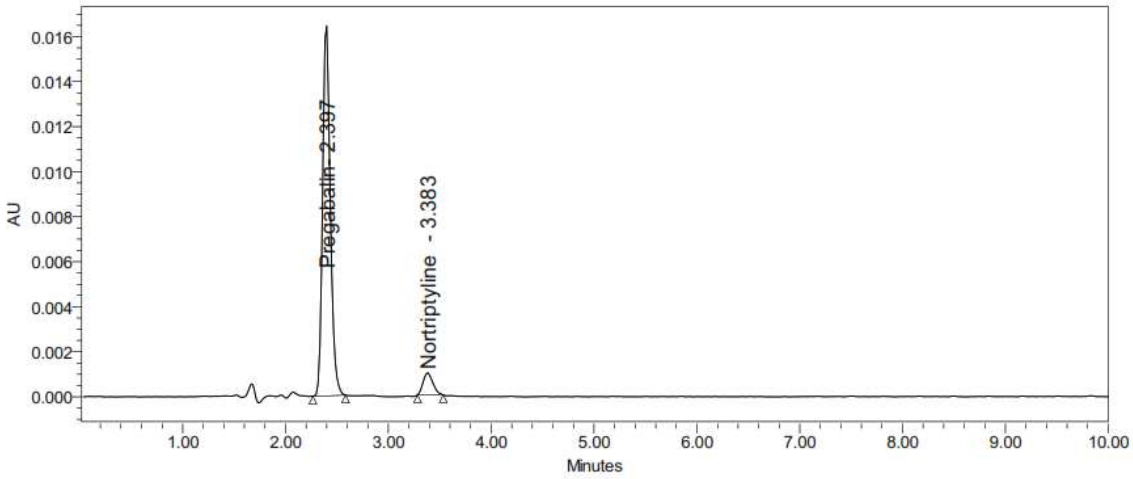

FIGURE 7: CHROMATOGRAM FOR LINEARITY-3

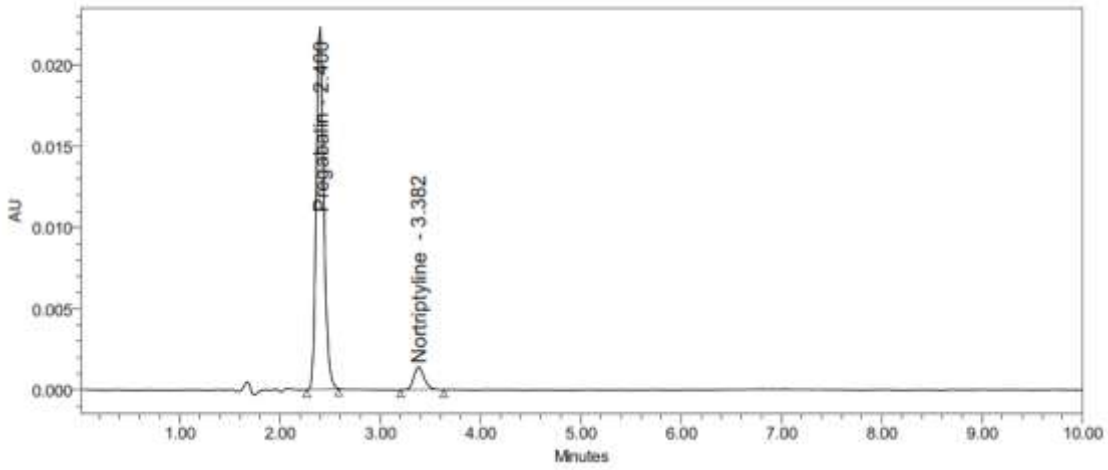

FIGURE 8: CHROMATOGRAM FOR LINEARITY-4

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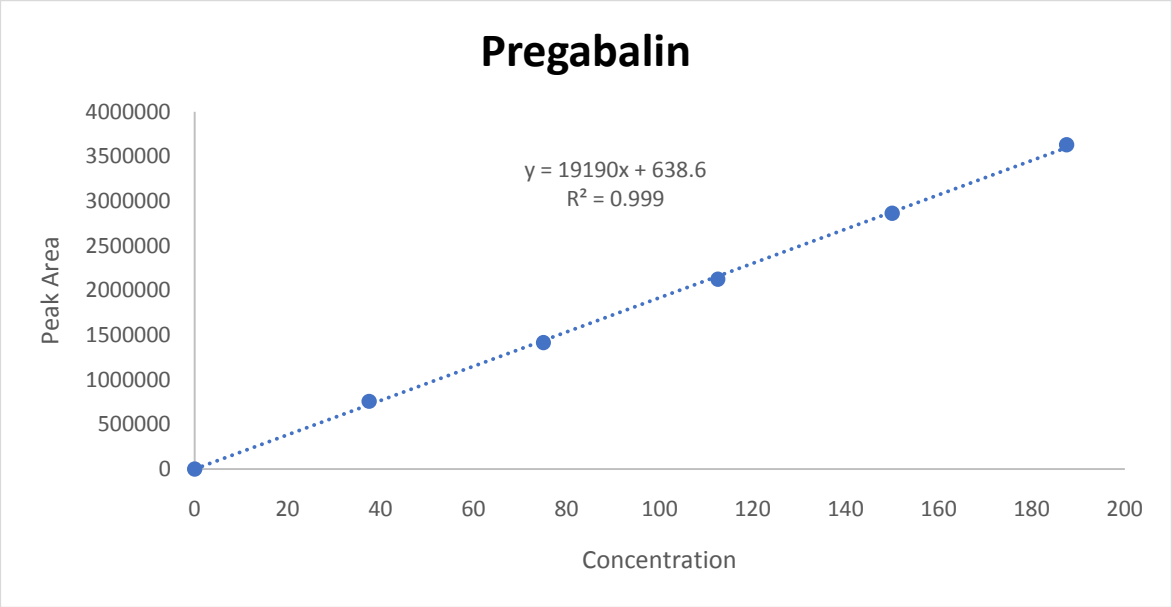

FIGURE 10: CALIBRATION GRAPH FOR PREGABALIN

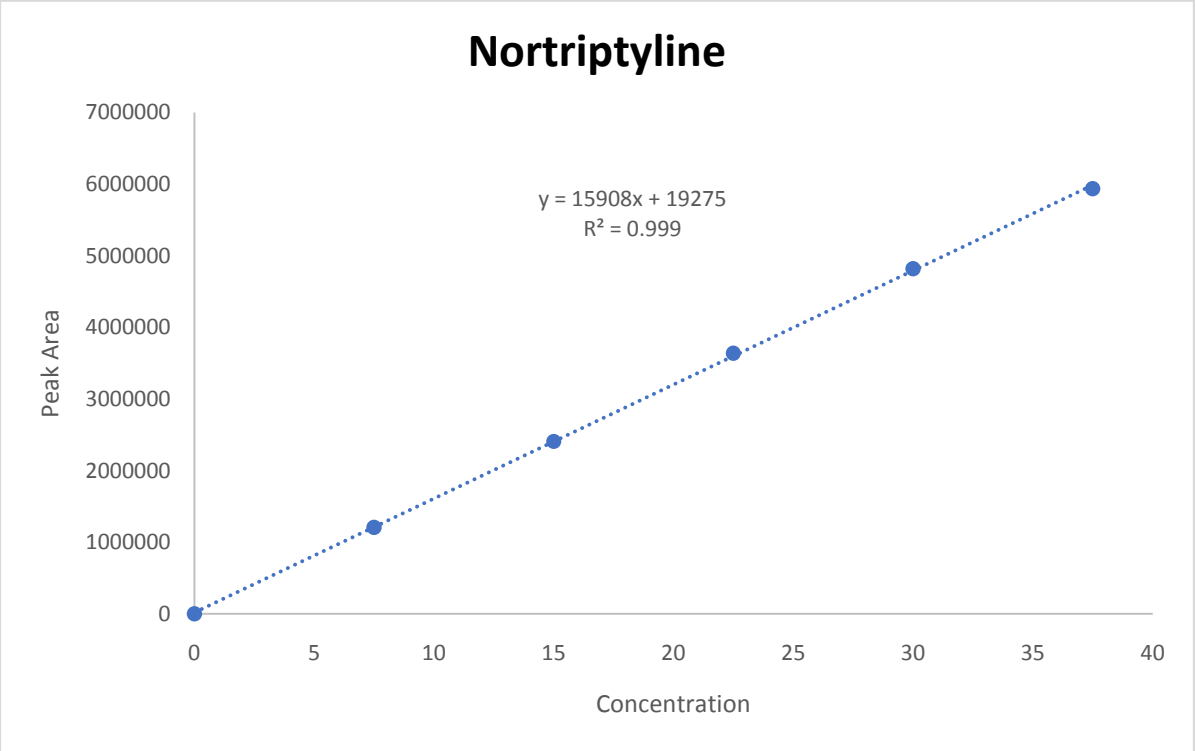

FIGURE 11: CALIBRATION GRAPH FOR NORTRIPTYLINE

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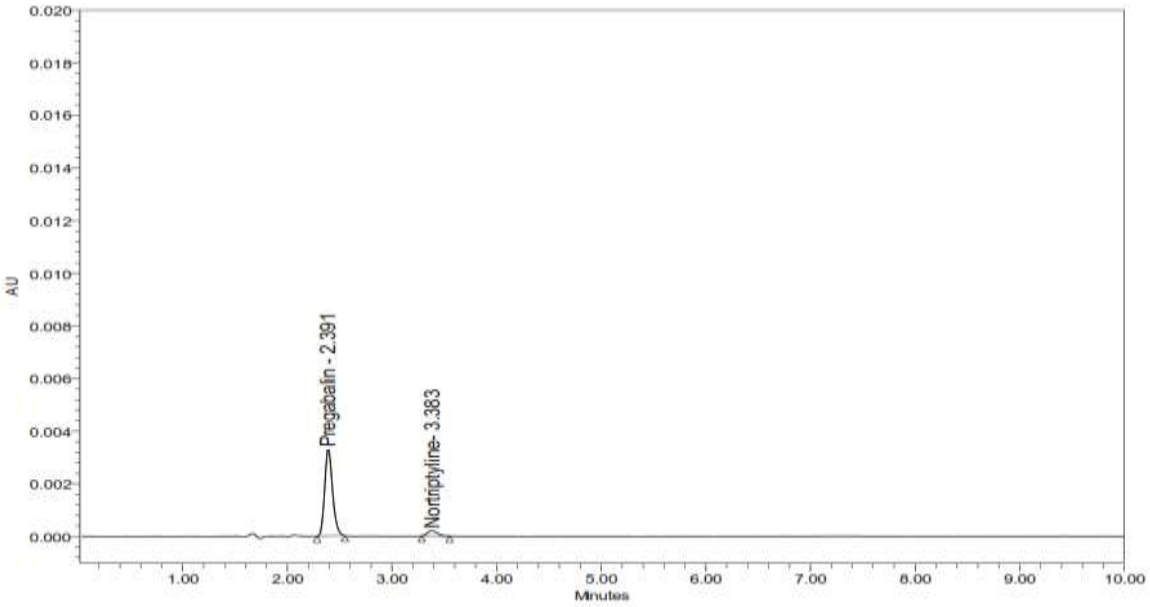

FIGURE 32: CHROMATOGRAM OF PREGABALIN, NORTRIPTYLINE SHOWING LOD

TABLE 16: RESULTS OF LOD

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Pregabalin	55	164	2.98
Nortriptyline	55	162	2.95

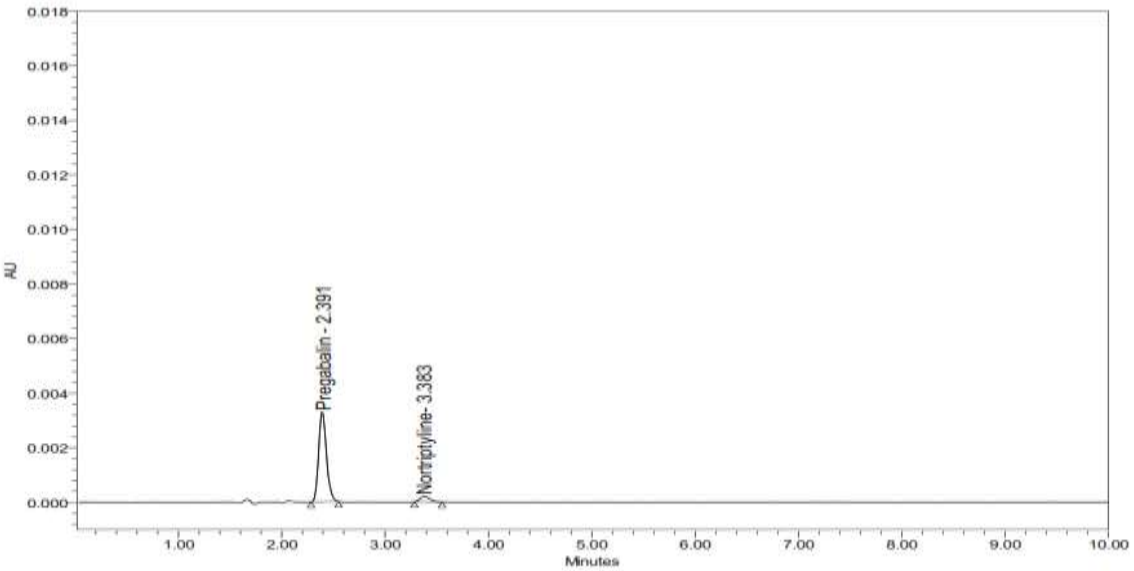

FIGURE 33: CHROMATOGRAM OF PREGABALIN, NORTRIPTYLINE SHOWING LOQ

TABLE 17: RESULTS OF LOQ

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Pregabalin	55	548	9.96
Nortriptyline	55	549	9.98

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IV. CONCLUSION

The accuracy limit is the percentage recovery should be in the range of 97.0% - 103.0%. The total recovery was found to be 99.09% and 100.73% for Pregabalin and Nortriptyline. The validation of developed method shows that the accuracy is well within the limit, which shows that the method is capable of showing good accuracy and reproducibility.

The acceptance criteria for LOD and LOQ is 3 and 10.The LOD and LOQ for Pregabalin was found to be 2.98 and 9.96 and LOD and LOQ for Nortriptyline was found to be 2.95 and 9.98.

The robustness limit for mobile phase variation and flow rate variation are well within the limit, which shows that the method is having good system suitability and precision under given set of conditions. The developed method can be used in the routine drug analysis of pharmaceutical formulations.

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