Research Article
AMERICAN JOURNAL OF PHARMACY AND HEALTH RESEARCH
www.ajphr.com
2015, Volume 3, Issue 4
ISSN: 2321–3647(online)

# Development and Validation of New RP-HPLC Method for Simultaneous Determination of Nortriptyline and Gabapentin in Combined Dosage Form

**BV.Sreenivasulu[1] V.Siva RamaKrishna[2]** and **C.Ramababu[2]***
*1.Department of Biochemistry, Acharya Nagarjuna University, Guntur, AP, India.*
*2.Department of Chemistry, Acharya Nagarjuna University, Guntur, AP, India*

## ABSTRACT

A simple, precise, rapid and accurate reverse phase high performance liquid chromatography method was developed for simultaneous estimation of Nortriptyline and Gabapentin in dosage form. Chromatographic separation was performed on Zorbax C18, (250 X 4.6mm, 5µm) column, with mobile phase comprising of mixture of buffer (pH 4.2) and acetonitrile in the ratio of 70:30v/v, at the flow rate 1.0 ml/min. The detection was carried out at 253nm. The retention times of Nortriptyline and Gabapentin were found to be 2.866 and 3.629 mins respectively with a run time of 6 mins, respectively. As per ICH guidelines the method was validated for linearity, accuracy, precision, limit of detection and limit of quantitation, robustness and ruggedness. Linearity of Nortriptyline was found in the range of 60-180µg/mL and that for Gabapentin was found to be 300-900µg/mL. The correlation coefficient for Nortriptyline and Gabapentin were 1.000 and 0.9999 respectively. The LOD values for nortriptyline and gabapentin were found to be 0.0013µg/mL and 0.007µg/mL, respectively and the LOQ values 0.004µg/mL and 0.024µg/mL respectively.

**Keywords:** RP-HPLC Method Development, Nortriptyline and Gabapentin

*Corresponding Author Email: sreenubv@gmail.com
Received 23 March 2015, Accepted 07 April 2015

Please cite this article as: Rambabu C *et al.*, Development and Validation of New RP-HPLC Method for Simultaneous Determination of Nortriptyline and Gabapentin in Combined Dosage Form. American Journal of Pharmacy & Health Research 2015.

## INTRODUCTION

Nortriptyline hydrochloride [3-(10, 11-dihydro-5H-dibenzo [a,d] cyclohepten- 5-ylidene)-N-methyl-1-propanamine] [Figure.1.a], the *N*-demethylated active metabolite of amitriptyline, is a dibenzocycloheptene-derivative tricyclic antidepressant (TCA)[1] contains a tricyclic ring system with an alkyl amine substituent on the central ring. The antidepressant effects of TCAs are thought to be due to an overall increase in serotonergic neurotransmission. TCAs also block histamine-$H_1$ receptors, $\alpha_1$-adrenergic receptors and muscarinic receptors, which accounts for their sedative, hypotensive and anticholinergic effects (e.g. blurred vision, dry mouth, constipation, urinary retention), respectively.

Gabapentin (GBP) [Figure.1.b] is described as 1-(aminomethyl)cyclohexanacetic acid) with a molecular formula of $C_9H_{17}NO_2$ and a molecular weight of 171.24, is a new antiepileptic drug which is a structural analogue of neurotransmitter of gamma amino butyric acid (GABA) is used for the treatment of partial onset seizures with or without secondary generalized tonic-clonic convulsions in clinical practice[2,3].

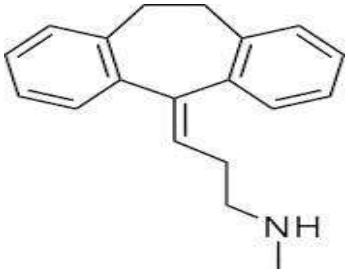


**Figure.1.b: Structure of Nortriptyline**

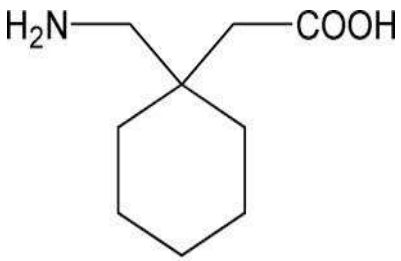


**Figure.1.b: Structure of Gabapentin**

From the literature review few analytical methods[4-10] were reported that for the assay of Gabapentin and Nortriptyline in either individually or in combination with other drugs. To date, only one RP-HPLC method[11] was reported for simultaneous estimation of these two drugs respectively. It was felt necessary to develop a simple, inexpensive, sensitive RP-HPLC method for the determination of nortriptyline and gabapentin in combined dosage forms.

In this paper a brief description was given by the author on the development and validation of new sensitive reverse phase liquid chromatographic method for the assay of nortriptyline and gabapentin in combined dosage forms in accordance ICH norms.

## MATERIALS AND METHOD

**Materials:**

Reference samples of gabapentin and nortriptyline were procured as gift samples from Wockhardt Ltd.,India. Tablet dosage form in the brand name, Gabain–NT tablets (Gabapentin-

400mg + nortriptyline-10mg) of Aristo pharmaceuticals was procured from local pharmacy.Potassium dihydrogen orthophos-phate and dipotassium hydrogen phosphate were of AR grade and acetonitrile and water of HPLC grade were used.

**Instrumentation:**

HPLC system (waters 2489, Milford, USA) equipped with quaternary gradient pump (TM 600), rheodyne manual injector with 20µl loop, column ( Zorbax C18 column) oven and UV detector was employed for analysis. The chromatographic data was acquired using Empower software.

**Mobile phase preparation:**

Accurately weighed and transferred 2.72g of potassium dihydrogen orthophosphate and 0.525g of di potassium hydrogen phosphate to a 1000 ml volumetric flask, 300 ml water was added and the volume was made up to 1000 ml with water. The pH was adjusted to 4.2. This buffer and acetonitrile were mixed in the ratio of 70:30, v/v and was used as mobile phase in the present assay. Prior to use it was filtered through a 0.45 µm membrane filter and degassed for further use.

**Diluent preparation:**

Water of HPLC grade was used as a diluent.

**Standard preparation:**

Standard stock solution containing nortriptyline (100mcg/ml) and gabapentin (1000mcg/ml) was prepared by transferring accurately weighed 10mg of nortriptyline and 100mg gabapentin working standard powder into a 100ml volumetric flask. 40ml of diluent was added to the flask, sonicated and cooled to room temperature. This solution was diluted to the mark with same diluent. Working standard solutions containing 60-180µg/mL of nortriptyline and 300-900µg/mL of gabapentin were prepared by pipetting aliquots of this stock solution into a 10ml volumetric flask and diluted up to the mark with the same diluent.

**Assay of formulation:**

Twenty tablets of Gabain–NT (Label claim;Gabapentin-400mg and nortriptyline-10mg) were accurately weighed & powdered. The quantity equivalent to 10mg of nortriptyline and Gabapentin-100mg were transferred to 100ml amber colored volumetric flask and to this 60ml distilled water was added & sonicated for 15 min at room temperature & then diluted to the mark with distilled water. The sample solution was filtered through whatmann filter paper prior to use. Each of the solutions (20µL) was then injected six times into the column. From the peak areas, the drug content in tablets were quantified using the regression equation obtained from pure sample and the relevant results are shown in Table: 6.

# RESULTS AND DISCUSSION:

**Development and optimization of the HPLC method:**

The analytical conditions for the proposed method were selected, basing on the chemical nature of nortriptyline and gabapentin.

Initial spectroscopic analysis of compounds showed that nortriptyline and gabapentin showed a maximum UV absorbance (λmax) at 250nm, 254nm respectively. Therefore, the chromatographic detection was performed at 253nm using a photo diode array detector as both the compounds showed good response at this wave length. The column selection has been done on the basis of back pressure, resolution, peak shape, theoretical plates and day-to-day reproducibility of the retention time and resolution between nortriptyline and gabapentin peak. After evaluating all these factors, Zorbax C18 column; 250 mmx4.6 mm I.D; particle size 5µm) was found to be suitable as it gave satisfactory results. The selection of buffer based on chemical nature of both the drugs. The acidic pH range was found suitable for solubility, resolution, stability, theoretical plates and peak shape of both components. Best results were obtained with phosphate buffer (4.2) for nortriptyline and gabapentin. Acetonitrile was chosen as organic constituent of mobile phase, to reduce the longer retention time and to attain good peak shape. Preliminary trials using different composition of mobile phases consisting of buffer (4.2) and acetonitrile in the ratio of 60:40 v/v and 50: 50 v/v, that did not give good peak shape for nortriptyline and gabapentin.

Finally, the best separation and resolution of nortriptyline and gabapentin is achieved by fixing mobile phase composition consisting of a mixture of buffer (4.2) and acetonitrile in the ratio of 70:30 v/v achieved. Under these conditions nortriptyline and gabapentin were eluted at 2.886 and 3.629, minutes respectively with a run time of 6 min. Optimized mobile phase proportion provided good resolution between nortriptyline and gabapentin and the flow rate was maintained at 1.0ml/min and the eluents were monitored at 253nm. The typical HPLC chromatogram for simultaneous estimation of nortriptyline and gabapentin standard by using the aforementioned chromatographic conditions is represented in Figure.2. System suitability results of the method are presented in Table.1.

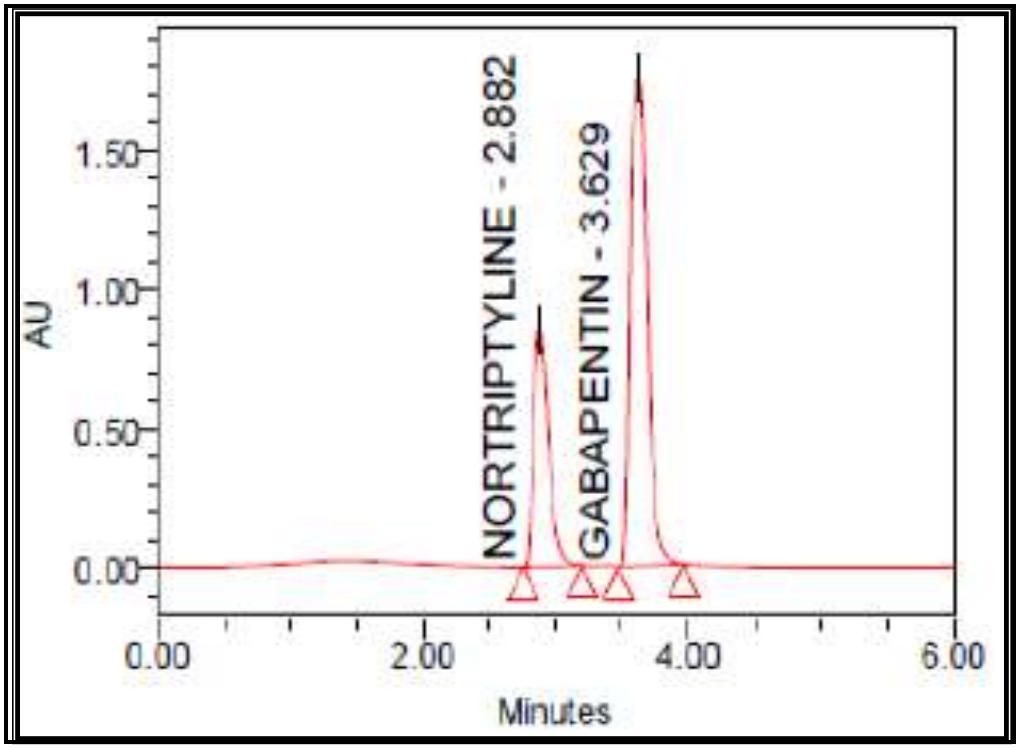


**Figure: 2- Typical HPLC Chromatogram Showing the Peaks of Nortriptyline and Gabapentin**

**METHOD VALIDATION:**

**System suitability:**

A system suitability test of the chromatographic system was performed before each validation run. Five replicate injections of standard preparation were injected and the column efficiency, resolution and peak asymmetry were calculated for the standard solutions (Table.1). The values obtained demonstrated the suitability of the system for the analysis of this drug combinations, system suitability parameters may fall within ± 3.0 % standard deviation range and % RSD less than 2.0 during routine performance of the method.

**Table.1: System Suitability Parameters**

| Parameters | Nortriptyline | Gabapentin |
|---|---|---|
| Retention time | 2.886 | 3.629 |
| USP Plate count | 3611 | 3694 |
| USP Tailing | 1.432 | 1.542 |
| Linearity Range (µg /ml) | 60-180 | 300-900 |
| Limit of Detection (LOD) (µg /ml) | 0.0013 | 0.007 |
| Limit of Quantitation (LOQ) (µg /ml) | 0.004 | 0.024 |

**Linearity of detector response:**

The standard curve was obtained in the concentration range of 60-180µg/ml for nortriptyline and 300-900µg/mL for gabapentin. The linearity of this method was evaluated by linear regression

analysis. Slope, intercept and correlation coefficient [r2] of standard curve were plotted and calculated and are given in Figure: 3.a & Table: 2. for nortriptyline and Figure:3.b & Table:2 for gabapentin demonstrating the linearity of the proposed method.

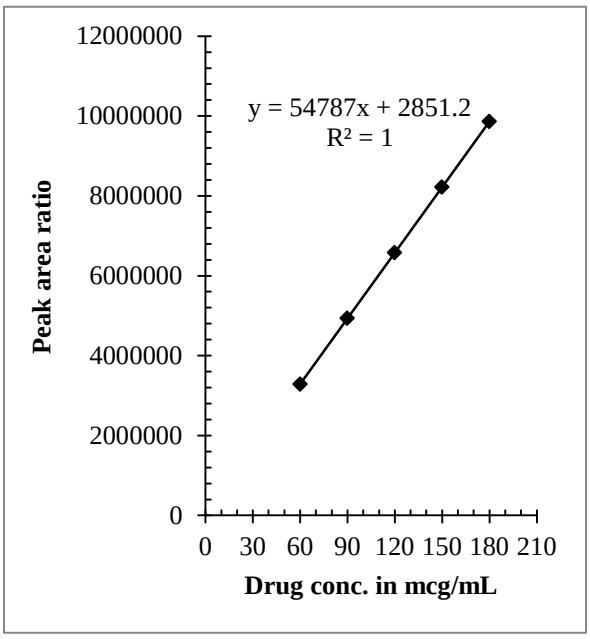


**Figure.3.a: Linearity plot of Nortriptyline**

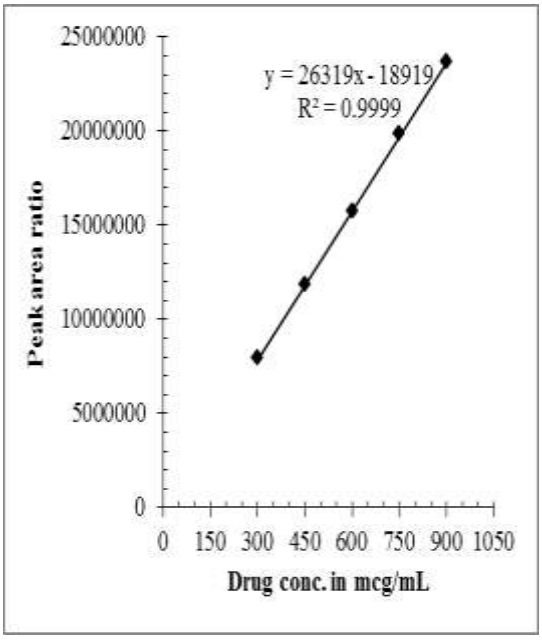


**Figure. 3.b: Linearity plot of Gabapentin**

**Table.2: Linearity studies of Nortriptyline and Gabapentin by the proposed method**

| Linearity study for nortriptyline | | | Linearity study for gabapentin | | |
|---|---|---|---|---|---|
| **% Level (approx.)** | **Conc. µg/ml** | **Area** | **% Level (approx.)** | **Conc. µg/ml** | **Area** |
| 50 | 60 | 3286068 | 50 | 300 | 7894616 |
| 75 | 90 | 4938875 | 75 | 450 | 11826470 |
| 100 | 120 | 6578171 | 100 | 600 | 15705853 |
| 125 | 150 | 8219540 | 125 | 750 | 19777554 |
| 150 | 180 | 9863780 | 150 | 900 | 23658461 |
| Slope | | 54787 | Slope | | 26319 |
| Intercept | | 2851.2 | Intercept | | 18919 |
| RSQ(r2) | | 1.0000 | RSQ(r2) | | 0.9999 |
| LOD | | 0.0013 | LOD | | 0.007 |
| LOQ | | 0.004 | LOQ | | 0.024 |

**LOD & LOQ:**

The limit of detection and limit of quantification were evaluated by serial dilutions of nortriptyline and gabapentin stock solution in order to obtain signal to noise ratio of 3:1 for LOD and 10:1 for LOQ. The LOD values for nortriptyline and gabapentin were found to be 0.0013µg/mL and 0.007µg/mL, respectively and the LOQ values 0.004µg/mL and 0.024µg/mL and are reported in Table.2 respectively.

**Precision:**

Data obtain from precision experiments are given in Table 1 for intraday and interday precision study for both nortriptyline and gabapentin. The RSD values for intra-day precision study and inter-day precision study was < 2.0 % for nortriptyline and gabapentin confirming that the developed RP-HPLC method was precise. The results of accuracy studies are summarized in Table. 3.

**Table.3 :Precision Data of Nortriptyline and Gabapentin**

| | Nortriptyline Peak area | Gabapentin Peak area |
|---|---|---|
| | 6578066 | 15749140 |
| | 6574421 | 15765459 |
| | 6579352 | 15779528 |
| | 6574187 | 15791995 |
| | 6576920 | 15778096 |
| | 6573077 | 15746303 |
| Average* | 6576004 | 15768420 |
| SD* | 2476.882 | 18124.24 |
| %RSD* | 0374 | 0.110 |

*Average of six determinations

**Accuracy:**

Known amounts of standard nortriptyline and gabapentin added to pre-analyzed samples and were subjected to the proposed HPLC method at 50%, 100% and 150% to evaluate the degree of accuracy. The results of recovery studies are reported in Table.4.a&b. From the data reported in Table.4.a&b the mean recovery data obtained for each level as well as for all levels combined were within the acceptance criteria revealing good accuracy.

**Table.4a: Recovery Studies of the proposed RP-HPLC method**

| Concentration of Nortriptyline (µg/ml) | Amount added (µg/ml) | Total amount (µg/ml) | Amount found (µg/ml) | % Recovery | Mean |
|---|---|---|---|---|---|
| 120 | 20 | 140 | 139.89 | 99.92 % | 99.94 % |
| 120 | 40 | 160 | 159.99 | 99.93 % | |
| 120 | 60 | 180 | 179.95 | 99.97 % | |

**Table.4b: Recovery Studies of the Proposed RP-HPLC Method**

| Concentration of Gabapentin (µg/ml) | Amount added(µg/ml) | Total amount (µg/ml) | Amount found(µg/ml) | % Recovery | Mean |
|---|---|---|---|---|---|
| 900 | 100 | 1000 | 999.89 | 99.98 % | 99.95% |
| 900 | 200 | 1100 | 1098.99 | 99.90% | |
| 900 | 300 | 1200 | 1199.98 | 99.99 % | |

**Robustness:**

To evaluate the robustness of the developed RP-HPLC method, small deliberate Variations in the optimized method parameters were done. The effect of change in flow rate and mobile phase ratio on the retention time and tailing factor were studied. The results of this robustness study were reported in Table.5. The values for proposed method are well within acceptance limits. It was also observed that there were no marked changes in chromatograms, which demonstrated that the developed method was robust in nature.

**Table.5: Recovery Studies of the Proposed RP-HPLC Method**

| Robust conditions | | Nortriptyline | | | Gabapentin | | |
|---|---|---|---|---|---|---|---|
| | | Theoretical Plates | RT | Peak Area | Theoretical Plates | RT | Peak Area |
| Flow rate | 0.8 ml/min | 2744 | 2.888 | 6566165 | 4030 | 3.668 | 15797961 |
| | 1.2 ml/min | 2912 | 2.882 | 6593931 | 4095 | 3.768 | 15804738 |
| Temp. | 40$^{o}$C | 2938 | 2.880 | 6574779 | 4070 | 3.573 | 15864008 |
| | 45$^{o}$C | 2795 | 2.799 | 6575245 | 4148 | 3.659 | 15767369 |

**Assay of formulation:**

From the peak areas, the content of nortriptyline and gabapentin in tablet formulation of Gabain–NT were quantified using the regression equation obtained from pure sample and the relevant results are shown in Table: 6. The amount of nortriptyline and gabapentin in Gabin NT formulation obtained with the proposed method are between 99.84.8% and 99.94%, and were within the acceptance level of 90% to 110% respectively revealing that the developed method can be conveniently used for the assay determination of nortriptyline and gabapentin in combined dosage forms.

**Table.6: Analysis of Marketed Tablets by the Proposed Method**

| Market formulation | Drug composition | Label claim | Quantity found* | %Assay |
|---|---|---|---|---|
| GABIN -NT | Gabapentin | 400 | 399.76 | 99.94 |
| | Nortriptyline | 10 | 9.984 | 99.84 |

*Average of six determinations

## CONCLUSION

The developed RP-HPLC method was proved to be simple, fast and reliable. The developed method offers several advantages in terms of simplicity in mobile phase, isocratic mode of elution and sample preparation steps and comparative short run time makes the method specific, repeatable and reliable for its intended use in simultaneous determination of nortriptyline and gabapentin in tablet dosage form. From the above citations it can therefore be concluded that

"the proposed RP-HPLC method developed by the author for the assay of Nortriptyline and Gabapentin can be used in small laboratories with very high accuracy and precision".

## ACKNOWLEDGEMENT:

The authors are thankful to Bio Lee.Labs-Hyderabad, Andhra Pradesh and Dept. of Chemistry, Acharya Nagarjuna University, Guntur, AP, India for providing necessary for providing laboratory facilities to carry out this research and the authors greatly acknowledge Wockhardt Ltd.,India.for providing the gifted samples.

## REFERENCES:

1. Prochazka AV, Weaver MJ, Keller RT, Fryer GE, Licari PA, Lofaso D. A randomized trial of nortriptyline for smoking cessation. Arch Intern Med. 1998; 158(18):2035-9.
2. Goa KL, Sorkin EM. Gabapentin. A review of its pharmacological properties and clinical potential in epilepsy, Drugs.1993; 46 (3): 409–27.
3. Elwes RD, Binnie CD. Clinical pharmacokinetics of newer antiepileptic drugs. Lamotrigine,vigabatrin, gabapentin and oxcarbazepine. Clin Pharmacokinet.1996; 30:403–415.
4. Sameer AM, Basavaiah K. Highly sensitive spectrophotometric method for the determination of gabapentin in capsules using sodium hypochloride. Turkish J Pharma Sci.2012; 9(2):113-126.
5. Pavani DT, Karimulla SK, Rajesh B, Gayatri P, Vasanth PM, Ramesh M. Validation Of rapid and sensitive spectrophotometric method for the determination of gabapentin in tablet dosage forms. Int J Biological Pharma Res 2012;3(6):800-803.
6. Lakshmi B. RP-HPLC Method development for the quantification of Gabapentin in Formulations. Int J Sci Technol 2012;2(1):84-92.
7. Syed SQ. Validation of an Isocratic HPLC assay of Gabapentin in pharmaceutical formulations and stress test for stability of drug substance. Scholars Research Library. 2011;3(4):342-350.
8. Udaykumarrao B, Anna PN. Determination of gabapentin in bulk drug and in pharmaceutical dosage form by HPLC method. J. Chem. Soc. 2009;54(4):424-427.
9. Victoria FS, Maria KN, Ioannis N. Development of an HPLC method for the monitoring of tricyclic antidepressants in biofluid J Separation Sci 2102;30(1):45-52.

10. Safwan A, Nuha K. Simultaneous determination of nortriptyline hydrochloride and fluphenazine hydrochloride in microgram quantities from low dosage forms by liquid chromatography–UV detection. J Pharma Analysis.2012; 2(6):437-442.
11. M.NaveenKumar, D. SaiMalakondaiah, G.Usha Sree, A. Ajitha, V. Uma Maheshwara Rao. Int J Pharma Res Analysis.2015;5(1):13-17.