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Research

RP-HPLC and UV-Detection Method Development for the Simultaneous Quantification of Gabapentin and Nortriptyline in Bulk and Tablet Dosage Form

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Abstract

A Reverse phase high performance liquid chromatography with UV detection method is developed for the simultaneous quantification of Gabapenetin and Nortriptyline in bulk and in its combined tablet dosage form. The chromatographic separation was performed on YMC C8 column (150mm×4.6mmI.D 5 µm particle size) using isocratic elution.

The optimized mobile phase consists of 0.1% the orthophosphoric acid and methanol (60:40 *v/v*).The eluted analytes are monitored at 238 nm wavelength using a UV detector. The developed method separates Gabapenetin and Nortriptyline within a run time of 6 min. The developed method was validated as per International Conference of Harmonization guidelines with respect to linearity, sensitivity (limit of detection and limit of quantification), selectivity, accuracy, precision and robustness. Linearity was observed in concentration range of 200-600 µg/ml for Gabapenetin and 5-15 µg/ml for Nortriptyline. The LOD was found to be 0.672 and 0.0117µg/ml for Gabapenetin and Nortriptyline respectively. The LOQ was found to be 2.240 and 0.0390 µg/ml for Gabapenetin and Nortriptyline, respectively. The range of percentage recovery of Gabapenetin and Nortriptyline was found to be 100.49-100.75% and 100.35-100.51%, respectively. The % RSD values of precision study for Gabapenetin were found to be 0.227% and for Nortriptyline it was foundtobe0.347%.All the validation parameter values are within the acceptable range. The developed and validated method was successfully applied to the determination of Gabapenetin and Nortriptyline in combined pharmaceutical dosage form without any interference from the excipients with good recovery, precision and accuracy.

Keywords: RP-HPLC, UV Detector, Gabapenetin, Nortriptyline, Isocratic Elution, Mobile Phase, Tablet dosage form

INTRODUCTION

The drugs are used in various forms in prophylactic or in therapeutic use (Bazaj, 1987); and the drugs are applied in some instances in rather small doses and they are often mixed with excipients as combinations. The assay of various dosage forms raises several special such as skillful sampling and the preparation of sample solutions. Hence standard techniques must be employed to ascertain the homogeneity of the sample before collecting for analysis. The methods of estimation of drugs are divided into physical, chemical, physicochemical and biological ones. Of them, physical and physicochemicalmethods are used the most. Physical methods of analysis involve the studying of the physical properties of a substance. They include determination of the solubility, transparency or degree of turbidity, color, density or specific gravity (for liquids), moisture content, melting, freezing and boiling points. Physicochemical methods (Day and Underwood, 1986; Willard*et al.,* 1986) are used to study the physical phenomena that occur as a result of chemical reactions. Among the physiochemical methods are optical (refractometry, polarimetry, emission and spectrophotometry, nephelometry or turbidometry), electrochemical (potentiometry, amperometry, colorimetry and polarography) and chromatography (column, paper, thin layer, gas liquid, high performance liquid methods are generally preferable In the present investigation, HPLC with UV detection has been used as toolsin the simultaneous quantification of the selected drugs.

HIGHPERFORMANCELIQUIDCHROMATOGRAPHY

High performance liquid chromatography (HPLC) (Daniel, 1987; Willard*et al.,*) is a special branch of column chromatography in which the mobile phase is forced through the column at highspeed. Therefore the analysis time is reduced by 1-2 orders of magnitude relative to classical column chromatography. The use of much smaller particles of the adsorbent becomes possible increasing the column efficiency substantially. The main components of the High Performance Liquid Chromatography

Mobile Phase

Mobile phases used for HPLC typically are mixtures of organic solvents and water or aqueous. Table 3 lists the physical properties of organic solvents likeAcetonitrile, Dioxane,Ethanol, Ethyl acetate, methanol, chloroform, Isopropanol, n-Propanol, THF and water commonly used for HPLC. Isocratic elution methods are preferable to gradient elution methods. Gradient elution methods will sometime be required when the molecules being separated have vastly different partitioningproperties. When a gradient elution method is used, care must be taken to make sure that all solvents are miscible.

Detectors

The detection with UV light absorbance offer both convenience and sensitivity for molecules. In a chromophore, the wavelength of detection for a molecule should be based on its UV spectrum in the mobile phase and not in pure solvents. The most selective wavelength for detecting a molecule is frequently the longest wavelength, maximum to avoid interference from the solvents, buffers and excipients. Other method of detection can be useful or required in some instances.

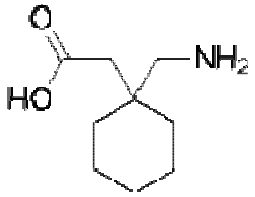
Validation of Analytical Data

The objective of the method validation process is to provide evidence that the method does what it is intended to (United States Food and Drug Administration, 2001; International Committee on Harmonization, 2005). While method validation process, all the variables of the method should be considered, including sampling procedure, sample preparation, chromatographic separation, and detection and dataevaluation. For chromatographic methods used in analytical applications there is more consistency in validation practice with key analytical parameters including (a) recovery (b) response function (c) Sensitivity (d) Precision (e) accuracy.

DRUGPROFILE: **Gabapenetin**

$C_9H_{17}N_2$ 1-(amino methyl) cyclohexane acetic acid is an anti-epileptic and an anticonvulsant medication. Gabapentin exerts its activity on chemicals and nerves in the body that are responsible for the cause of seizures and pain (Gabapentin, 2011). In adults, it is used to treat nerve pain caused by herpes virus or shingles. It is also used to treat restless legs syndrome. It is also used to treat seizures in adults and children aged 3 years (Johannessen & Ben- Menachem, 2006).

Structure of Gabapenetin



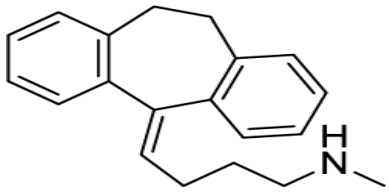
Mode of action

The exact mechanisms for the analgesic and anticonvulsant effects of gabapentin are not known. Gabapentin interacts with cortical neurons at auxiliary subunits of voltage sensitive calcium channels. Gabapentin increases the synaptic concentration of γ aminobutyric acid, enhances γ aminobutyric acid responses at non- synaptic sites in neuronal tissues, and reduces the release of mono-amine neurotransmitters (Davies et al., 2007; Patel & Dickenson, 2016).

Nortriptyline

$C_{19}H_{21}N$ 3-(5,6-dihydrodibenzo[2,1-b:2',1'-f][7]annulen-11-ylidene) N-methyl propane- 1-amine is a second generation dibenzocycloheptene derivative tricyclic antidepressant. It isN-demethylated active metabolite of amitriptyline. Nortriptyline is approved for the management of major depression. it is also used for the treatment of nocturnal enuresis, panic disorder, irritable bowel syndrome, migraine prophylaxis and chronic pain. International Association for the Study of Pain recommends Nortriptyline as a first-line medication for neuropathic pain (Dworkin et al., 2010).

Structure of Nortriptyline



SOLUBILITY

Soluble in ethanol and chloroform; sparingly soluble in methanol; Practically insoluble in water, ether, acetone and benzene**.**

Mode of action

Nortriptyline acts by blocking the nor-epinephrine presynaptic receptors. This results in blocking the reuptake of the neurotransmitter and raising the concentration in the synaptic cleft in the central nervous system. Nortriptyline also binds to α- adrenergic, histaminergic and cholinergic receptors. The treatment with Nortriptyline for a long time produces a down regulation of adrenergic receptors owing to the increased stimulation of these receptors (Brunton et al., 2010; National Institute of Mental Health, 2013).

Gabapenetin and Nortriptyline Combined Dosage Form

Gabapentin and Nortriptyline combined tablet dosage form is marketed with a brand name Nervi gab-NT. The tablet dosage form was manufactured by Jabs Biotech Pvt. Ltd. Nabha, Punjab, India. The combination of gabapentin and Nortriptyline is suggested to treat refractory neuropathic pain (Ian et al., 2009). They researchers propose that adding an antidepressant to an anticonvulsant used for pain relief may ease difficult-to-treat neuropathic pain.

Aim and Objective

To develop and validate a simple, sensitive, precise and accurate RP-HPLC with UV detection method for the simultaneous determination of gabapentin and Nortriptyline in bulk and combined tablet dosage form.

Method Development

Selection of mobile phase solvent Optimization of the HPLC parameters HPLC Performance calculations (Retention time, Theoretical Plates, Plates per Meter, Peak asymmetry)

Method Validation

Determination of the following parameters for the developed method System suitability studies Selectivity Linearity Sensitivity Precision Accuracy Robustness.

MATERIALSANDMETHODS

The reference standards of gabapentin and Nortriptyline were obtained as a gift sample from Lara Drugs Private Limited (Telangana, India). HPLC grade methanol was obtained from Merck specialties Ltd, Hyderabad. Analytical reagent grade orthophosphoric acid was purchased from Merck specialties Ltd, Hyderabad. Milli-Q water was used throughout the process Combined tablet dosage form (Nervi gab-NT, Jabs Biotech Pvt. Ltd. Nabha, Punjab, India) labeled to contain 200 mg of gabapentin and 5 mg of Nortriptyline was purchased from local pharmacy shop.

HPLC System: The Waters Alliance 2695 Module equipped with a 2998 PDA detector with Empower 2 software was used for the HPLC analysis.

Analytical column: The HPLC experimental conditions were optimized on the YMC C8 (150 mm x 4.6 mm internal diameter, 5 µm particle size) analytical column.

Mobile Phase: The mobile phase was prepared by mixing 0.1% orthophosphoric acid and methanol in the ratio of 60:40 (*v/v*). The mobile phase was also used diluents for the preparation of standard solutions.

Preparation of Sample Solution: Ten tablets were accurately weighed and crushed into a fine powder. The powderequivalentto200mgofGabapenetinand5 mg of Nortriptyline was taken into a 100 ml volumetric flask. About 50 ml of mobile phase was added, shaken for 5 min on rotary shaker followed by sonication for 20mins with intermediate shaking. The volumewas finallymade up to the mark with mobilephase. This solution was diluted aptly with mobile phase to get final sample concentration 400 µg/ml of gabapentin and 10µg /ml of Nortriptyline.

Calibration Curve: Working standard solutions equivalent to 200-600 µg/ml of Gabapentin and 5- 15 µg/ml of Nortriptyline were prepared by appropriate dilution of the stock standard solution with the mobile phase. Tenµl aliquot of each solution was injected into the column in triplicate. The mobile phase was pumped from the solvent reservoir to the column at a flow rate of 1.0 ml/min. The elute was monitored at 235 nm. The peak areas of all the concentrations of Gabapenetin and Nortriptyline were determined. Calibration curves were constructed by plotting peak area versus concentration.

Determination Of Gabapenetin And Nortriptyline In Pharmaceutical Dosage Form: Ten µl aliquot of solution prepared in the section "Preparation of sample solution" was injected automatically onto the column in triplicate. The chromatograms were recorded and the peak areas were determined. The nominal content of Gabapentin and Nortriptyline in the tablets were calculated using either the corresponding calibration curve or corresponding regression equation.

RESULTS AND DISCUSSIONS

Method Development

Two analytical columns, Inertsil ODS-3V and YMC C8, were initially tried during method development. The YMC C8 (150 mm x 4.6 mm internal diameter, 5 µm particle size) analytical column was selected for the separation and quantification of gabapentin and Nortriptyline, because it produced good separation of the selected drugs, well shaped symmetrical peaks with high resolution. The UV spectra of the gabapentin and Nortriptyline were assessed. It was observed from the UV spectra that gabapentin and Nortriptyline have considerable absorbance at 238 nm. Therefore, 238 nm was selected as the detection wavelength. In order to achieve satisfactory peak symmetry and separation with good resolution, various combinations of methanol, 0.1% orthophosphoric acid and phosphate buffers of different pH were tried systematically. Finally, a mobile phase consisting of 0.1% orthophosphoric acid and methanol in a ratio of 60:40 *v/v* was selected to achieve better resolution and acceptable peak symmetry. Flow rates between 0.5 and 1.5 ml/min were tried. Flow rate of 1.0 ml/min was observed to be adequate to get both the drugs eluted within less than 7min. The column temperature was set at 30°C. Under the optimized chromatographic conditions, the retention times for gabapentin and Nortriptyline were 2.818 min and 3.496 min, respectively. No interference was found among the two peaks. A typical chromatogram is shown in Figure 1.

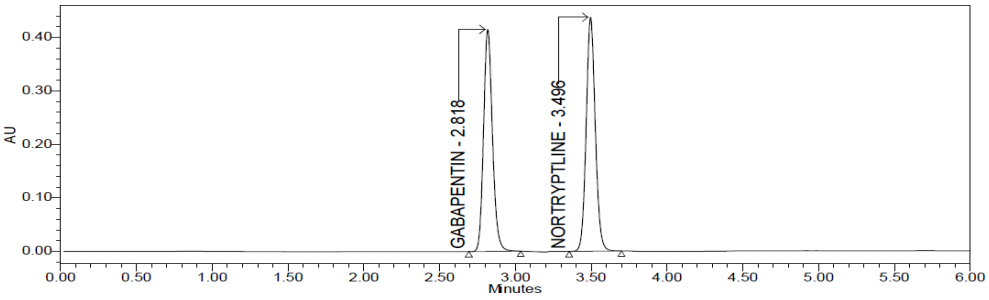


Fig 1: Standard chromatogram of Gabapentin and Nortriptyline

SYSTEM SUITABILITY

The system suitability was determined by injecting six replicates of the standard solutions and analyzing each active ingredient for its retention time, peak area, USP plate count, USP tailing and USP resolution. The system suitability results for a combined solution of 400µg/ml Gabapentin and 10µg/ml Nortriptyline revealed a %RSD of less than 1.0 % for both peak areas and retention times. All the system suitability parameters are within the recommended limit. This method meets the accepted requirements as shown in Table 1.

Table 1: System suitability

Parameters	Gabapentin	% RSD	Nortriptyline	% RSD	Recommended limit
Retention time (min)	2.826	0.121	3.503	0.136	RSD ≤2
Peak area	1687803	0.335	1820268	0.138	RSD ≤2
USP plate count	11370	0.516	16891	0.946	>2000
USP resolution	-	-	6.192	0.448	>1.5
USP tailing	1.164	0.470	1.104	0.496	≤2

[a]*Average of five values*

SELECTIVITY

The selectivity of the method was verified by comparing the chromatograms of standard mixed solution with the analytes concentration of 400 µg/ml (Gabapentin) and 40 µg/ml (Nortriptyline) with those of mobile phase blank, placebo and tablet sample solutions. The chromatograms are represented in Figures 5-8.There is no peak interference of blank and placebo at the retention time of gabapentin and Nortriptyline. The results indicated that there is no drug-drug interaction or drug- excipient interaction. Therefore, the method is selective for the determination of gabapentin and Nortriptyline in their combined pharmaceutical dosage form.

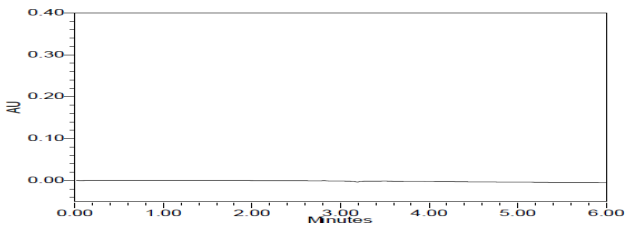


Fig 2: Chromatogramofmobile phase blank

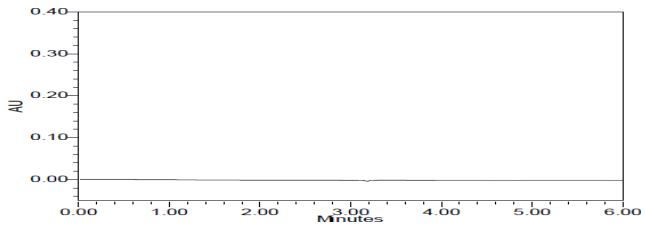


Fig3: Chromatogram ofplacebo

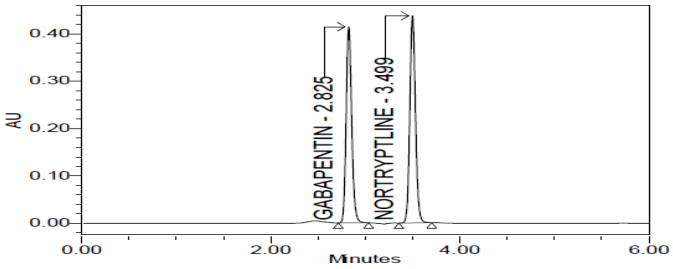


Fig 4: Chromatogram of Gabapenetin and Nortriptyline standard solution

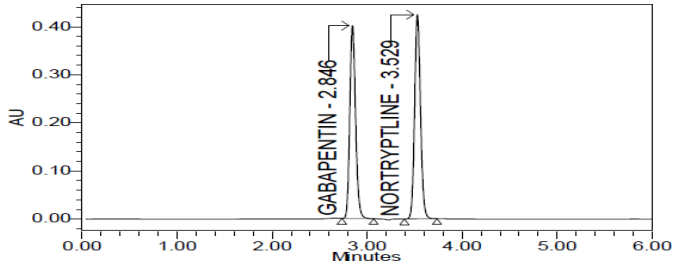


Fig 5: Chromatogram of Gabapenetin and Nortriptyline tablet sample solution

LINEARITY: linear relationship was established by plotting the peak area against the drug concentration. The relationship was found to be linear over the range 200–600 µg/ml and 5-15µg/ml for Gabapenetin and Nortriptyline, respectively. The results are summarized in Table 4. The results show that good correlation existed between the peak area and concentration of the studied drugs.

Table 4: Linearityofthemethod

Parameter	Gabapentin	Nortriptyline
Linearity (µg/mL)	200-600	5-15
Regression equation (Y=mx +c)	y=4218 x-1349	y=18246 x-1396
Slope(m)	4218	18246
Intercept(c)	-1349	-1396
Regression coefficient(R^2)	0.9998	0.9999

Y=Peak area, C=Concentration of drug in µg/ml

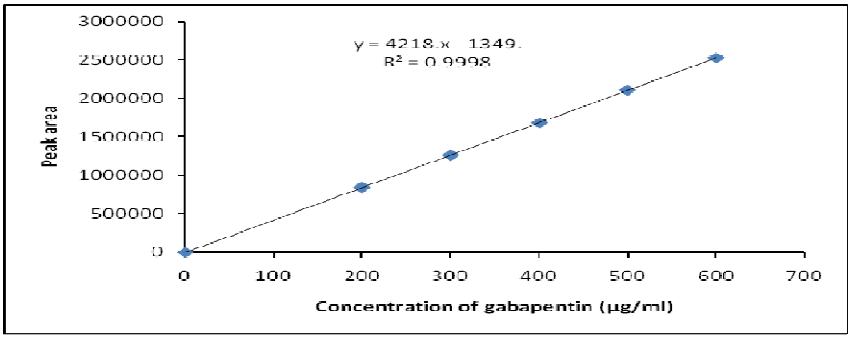


Fig 6: Linearity curve of Gabapenetin

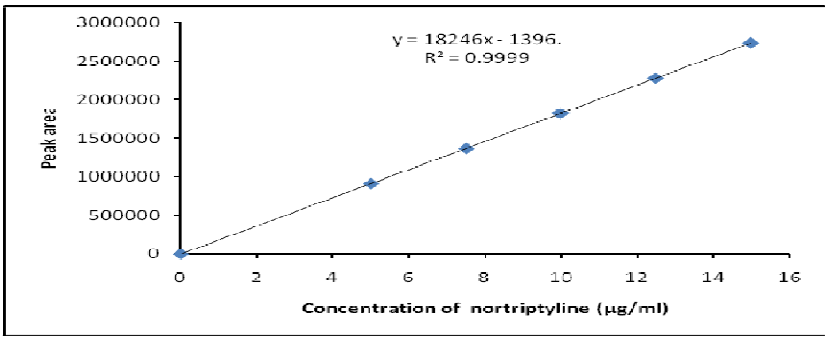


Fig 7: Linearity curve of Nortriptyline

SENSITIVITY: The sensitivity of the method was explored via measurement of the limit of detection (LOD) and limit of quantitation (LOQ) for gabapentin and Nortriptyline at a signal-to-noise ratio of 3 and 10, respectively. The LOD was found to be 0.672 and 0.0117 µg/ml for gabapentin and Nortriptyline, respectively. The LOQ was found to be 2.240 and 0.0390 µg/ml for gabapentin and Nortriptyline, respectively. The chromatogram of Gabapentin and Nortriptyline at LOD and LOQ levels are shown in Figures 11 and 12.

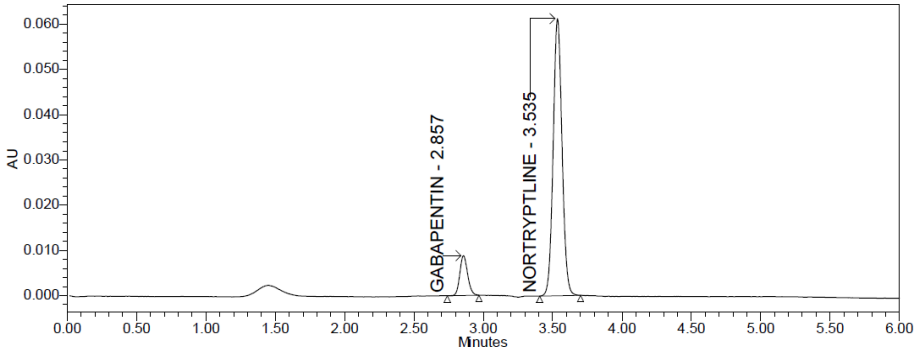


Fig 8: Chromatogram of Gabapenetin and Nortriptyline at LOD level

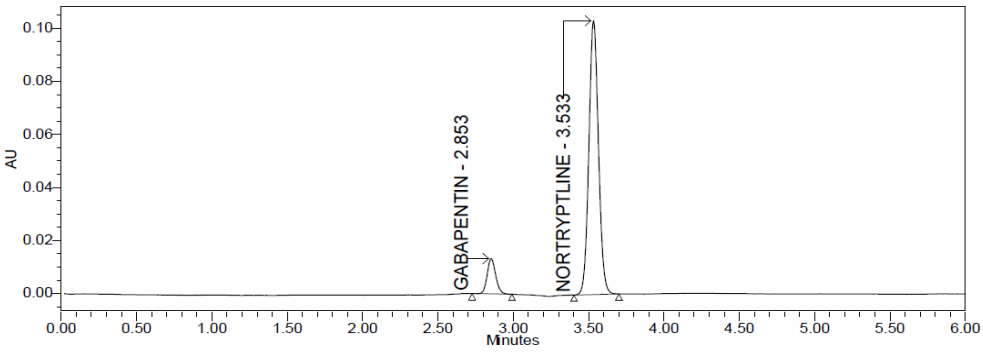


Fig 9: Chromatogram of Gabapentin and Nortriptyline at LOQ level

PRECISION: Precision of the method was assessed by injecting five standard solutions of Gabapenetin and Nortriptyline (within linearity range). Relative standard deviation of the peak area was then calculated to represent precision. The results are shown in Table 5.The relative standard deviation was found to be <1, which proves that the method is adequately precise. The chromatograms are shown in Figure 13.

Table 5: Precision of the method

Gabapentin		Nortriptyline	
Concentration (µg/ml)	**Peak area**	**Concentration (µg/ml)**	**Peak area**
400	1680151	10	1821756
400	1680588	10	1825750
400	1682709	10	1822480
400	1688879	10	1823469
400	1686939	10	1838233
400	1689164	10	1826303
Mean	**1684738**	**Mean**	**1826331**
%RSD	**0.227**	**%RSD**	**0.347**

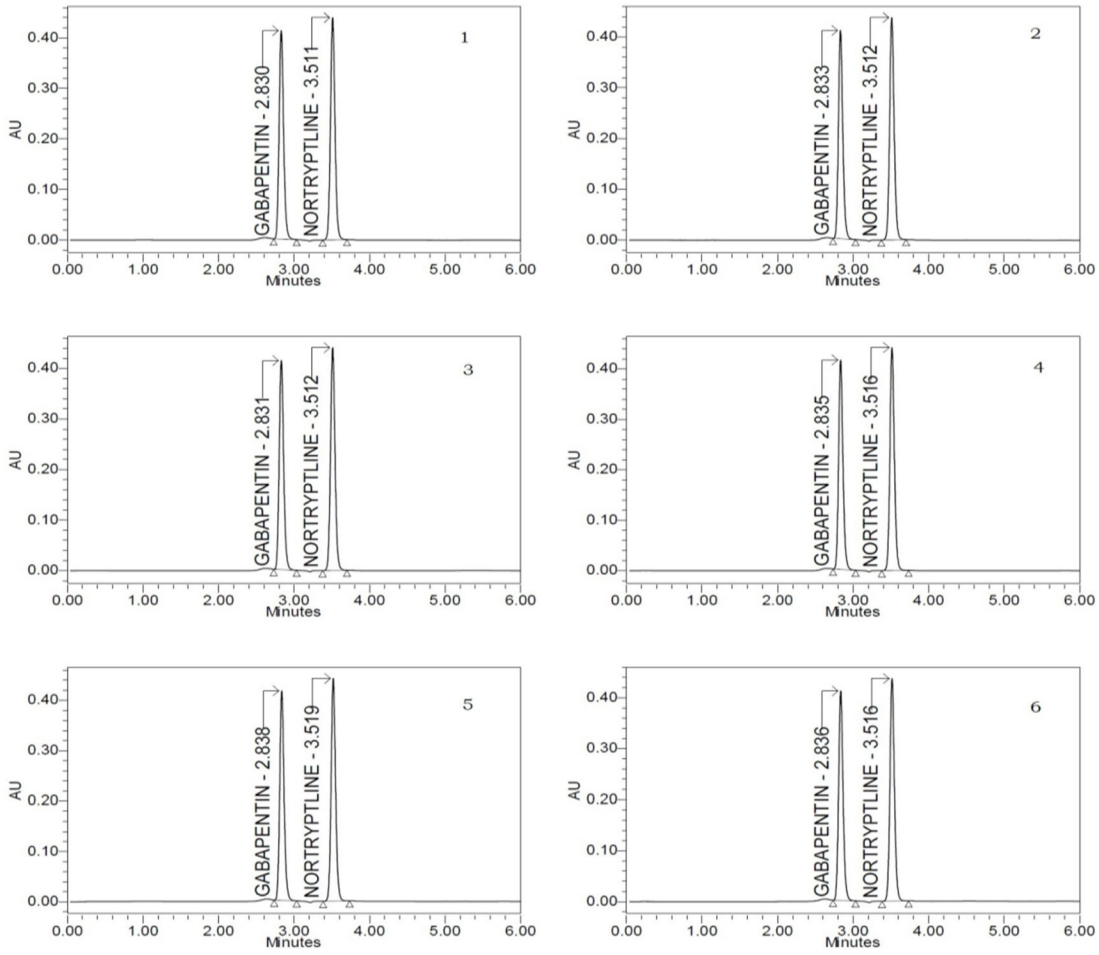


Fig10: Chromatogramsofmethodprecisionandaccuracy

ACCURACY: Accuracy of the method was assessed by injecting six standard solutions of Gabapentin and Nortriptyline (within linearity range). The percentage recoveries for gabapentin and Nortriptyline were calculated and are given in Table6. Thepercentage recovery value showed the method to be adequately accurate and suitable for the simultaneous determination of gabapentin and Nortriptyline.

Table 6: Accuracy of the method

Gabapentin		Nortriptyline	
Concentration (µg/ml)	**% Recovery**	**Concentration (µg/ml)**	**% Recovery**
400	99.05	10	99.08
400	99.07	10	99.30
400	99.20	10	99.12
400	99.56	10	99.17
400	99.45	10	99.98
400	99.58	10	99.33
Mean	**99.32**	**Mean**	**99.33**
SD	**0.242**	**SD**	**0.332**
%RSD	**0.243**	**%RSD**	**0.334**

RECOVERY STUDY: The accuracy of the proposed method was further established by means recovery study. The recovery study is performed through the standard addition technique, byadding a known amount of standard drug at three different levels (50%, 100% and 150%) to the pre-analyzed sample. Accuracy was expressed as percentage recovery in Table 7. The results indicating acceptable accuracy and absence of interference from the common excipients found in the tablet dosage forms. The chromatograms of Gabapentin and Nortriptyline at 100% presented in Figures.

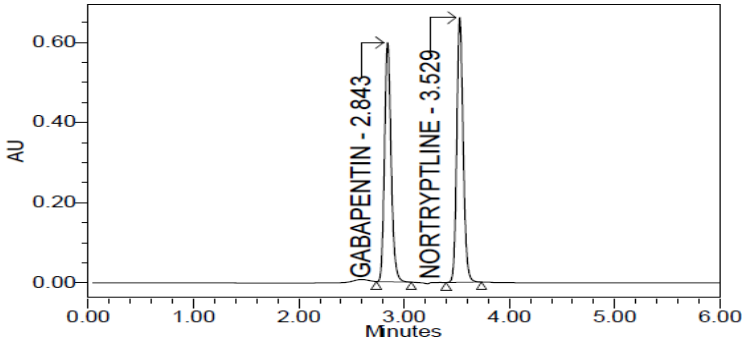


Fig11: Chromatogram of Gabapenetin and Nortriptyline at 100% level

ROBUSTNESS: The robustness of the proposed method was checked by studying the effect of deliberate changes in the flow rate of mobile phase (±0.1 ml/min) and column temperature (±2°C) on the chromatographic system suitability parameters. The robustness of the method was studied by using the standard solution with concentration400µg/mland10µg/ml of Gabapentin and Nortriptyline respectively**.**

CONCLUSION

In the present investigation a simple, fast and reliable RP-HPLC method was developed and validated for the simultaneous determination of gabapentin and Nortriptyline in bulk and combined tablet dosage form. The method validation showsa good performance with respect to linearity, sensitivity, accuracy, precision, selectivity and robustness. The developed and validated method was successfully applied for the simultaneous analysis of gabapentin and Nortriptyline in commercially available tablet dosage form. The common excipients present in the tablet dosage form did not interfere with the analysis. Therefore, the proposed method can be used in routine quality control laboratories.

REFERENCES

1. Alsante KM, Hatajik TD, Lohr LL, Sharp TR. Isolation and identification of process related impurities and degradation products from pharmaceutical drug Candidates. *American Pharmaceutical Review*, 2001, 4 (1), 70-78.
2. Ankit S, Charmy K, Nrupesh P. Concurrent estimation of gabapentin andnortriptyline hydrochloride in their combined dosage form using OPA-β- Mercaptoethanol derivatization by spectrophotometric and spectrofluorimetric methods. *Current Pharmaceutical Analysis*, 2016, 12 (4), Ahead of Print Article.
3. Antonella C, Alessandro M, Giovanni P, Enrico S, Marco V. Applications of high- performance liquid chromatography to metal carbonyl and cluster chemistry. *Chemical Reviews*, 1989, 89 (2), 407-418.
4. Atherden LM, Edr. Bentley and Drivers *Text Book of Pharmaceutical Chemistry,* 8th Edition, Oxford University Press, 1966, 4th Impression.
5. Bazaj ME. Edition, *Indian Pharmaceutical Guide,* Pamposh Publications, New Delhi, 1987.
6. Berek D, Russ A. Limited sample recovery in coupled methods of high-performance liquid chromatography of synthetic polymers. *Chemical papers,* 2006, 60 (3), 249–252.
7. Brunton L, Chabner B, Knollman B. Goodman and Gilman's *The Pharmacological Basis of Therapeutics,* Twelfth Edition. McGraw Hill Professional; 2010.CIMS,*CurrentIndexofMedicinalSpecialties,*(Jan-Mar)2010.
8. Daniel WA. Optical isomer separation by liquid chromatography. *Analytical Chemistry,* 1987, 59 (2), 84A–91A.
9. Davies A, Hendrich J, Van Minh AT, Wratten J, Douglas L, Dolphin AC. Functional biology of the alpha(2)delta subunits of voltage-gated calcium channels. *Trends in Pharmacological Sciences*, 2007, **28** (5), 220–228.
10. Day RA, Underwood AL. *Quantitative Analysis,* 5th Edition, Prentice Hall, New Delhi, 1986.
11. Deorge RF, Edtn, Wilson. Griswold's *Text Book of Organic Medicinal and Pharmaceutical Chemistry,* 8th Edn. Lippincott Company, 1982.
12. Definition and classification of Drug or Pharmaceutical Regulatory aspects of drug approval, 2013.
13. Dictionary.comUnabridged(v1.1),RandomHouse, Inc., 2007.
14. "Gabapentin". *The American Society of Health-System Pharmacists*. Retrieved 3April 2011.
15. International Committee on Harmonization. "*Validation of Analytical Procedures: Text and Methodology*," Q2(R1), Nov. 2005.