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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF GABAPENTINE AND NORTRYPTALINE

O.Anitha, P.Prapulla
Mother Teresa College of Pharmacy, Ghatkesar, NFC Nagar, Hyderabad, Telangana, India

Abstract:
A simple, precise, rapid, specific and accurate stability indicating reverse phase high performance liquid chromatography method was developed for simultaneous estimation of Gabapentin (GPT) and Nortriptyline (NTL) in pharmaceutical tablet dosage form. Chromatographic separation was performed on Agilent C8, (150 X 4.6mm, 5µm) column, with mobile phase comprising of mixture of buffer: (0.1M ammonium acetate) and methanol in the ratio of 70:30v/v, at the flow rate 1.0 ml/min. The detection was carried out at 254 nm. The retention times of GPT and NTL were found to be 2.66 and 3.58 mins respectively with a run time of 6 mins, theoretical levels for GPT and NTL were 8734 and 8648 respectively, with a resolution of 6.56. As per ICH guidelines the method was validated for linearity, accuracy, precision, limit of detection and limit of quantitation, robustness and ruggedness. Linearity of GPT was found in the range of 800-2400 µg/mL and that for CPG was found to be 20-60 µg/mL. The correlation coefficient for GPT and NTL were 0.999 and 1.000 respectively. The LOD values for GPT and NTL were 2.936 and 2.927 µg/mL respectively. The LOQ values for GPT and NTL were and 9.786 and 9.756 µg/mL respectively. This demonstrates that the developed method is simple, precise, rapid, selective, accurate and reproducible for simultaneous estimation of GPT and NTL tablet dosage form.

Keywords: *Gabapentin (GPT), Nortriptyline (NTL), RP-HPLC Method Development and Validation*

Corresponding author:
O.Anitha,
M. Pharmacy,
Department of pharmaceutical Analysis
Mother Teresa College of Pharmacy, Ghatkesar, NFC Nagar,
Hyderabad, Telangana, India
E-mail: anithaorsu0826@gmail.com

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INTRODUCTION:

Gabapentin is in the United States, gabapentin is officially indicated for the treatment of postherpetic neuralgia in adults and for the adjunctive treatment of partial-onset seizures, with or without secondary generalization, in patients 3 years of age and older.[1] In Europe, gabapentin is indicated for adjunctive therapy in the treatment of partial-onset seizures, with or without secondary generalization, in patients 6 years of age and older and as monotherapy in patients 12 years of age and older. It is also used in adults for the treatment of various types of peripheral neuropathic pain, such as painful diabetic neuropathy.[2]

The precise mechanism through which gabapentin exerts its therapeutic effects is unclear.[3] The primary mode of action appears to be at the auxillary α2δ-1 subunit of voltage-gated calcium channels (though a low affinity for the α2δ-2 subunit has also been reported).4 The major function of these subunits is to facilitate the movement of pore-forming α1 subunits of calcium channels from the endoplasmic reticulum to the cell membrane of pre-synaptic neurons.[5] IUPAC name is 2-[1-(aminomethyl)cyclohexyl]acetic acid. Molecular Formula is $C_9H_{17}NO_2$. Molecular weight is 171.2.

Nortriptyline is indicated for the relief of the symptoms of major depressive disorder (MDD).[6] Some off-label uses for this drug include treatment of chronic pain, myofascial pain, neuralgia, and irritable bowel syndrome.[7] Though prescribing information does not identify a specific mechanism of action for nortriptyline [8], 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. Tricyclic antidepressants do not inhibit monoamine oxidase nor do they affect dopamine reuptake.[9] As with other tricyclics, nortriptyline displays affinity for other receptors including mACh receptors, histamine receptors, 5-HT receptors, in addition to other receptors. IUPAC name is methyl (3- {tricyclo [9.4.0.0^{3,8}] pentadeca-1(15),3,5,7,11,13-hexaen-2-ylidene}propyl)amine. Molecular Formula is $C_{19}H_{21}N$. Molecular weight is 263.3.

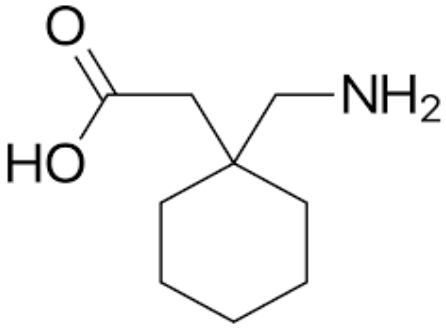

Figure 1: Structure of Gabapentin

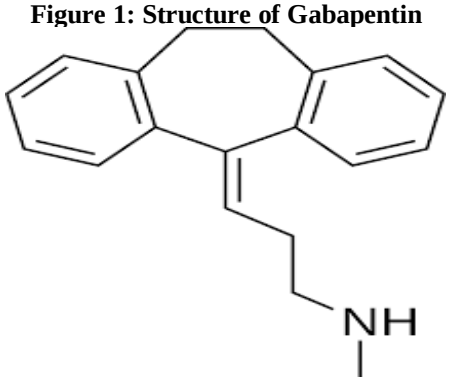

Figure 2: Structure of Nortriptyline

The literature survey revealed that There are really few approaches reported in the literary works for evaluation of Gabapentin and Nortriptyline alone or in combination with various other drugs in the pure form as well as drugs formulations by RP-HPLC [9-12]. In view of the demand for an appropriate, cost-effective RP-HPLC method for routine analysis of Gabapentin and Nortriptyline synchronized evaluation of in pharmaceutical dose type. Attempts were made to establish easy, precise, accurate as well as cost-efficient logical method for the estimate of Gabapentin and Nortriptyline. The recommended approach will be validated according to ICH guidelines. The objective of the recommended work is to establish a brand-new, simple, delicate, exact and economical logical method as well as recognition for the Synchronized evaluation of Gabapentin and Nortriptyline in pharmaceutical dose kind by utilizing RP-HPLC. To verify the established method based on ICH standards for the desired analytical application.

MATERIALS AND METHODS:

Chemicals and Reagents: Gabapentin and Nortriptyline were Purchased from Sun Pharma India Limited. NaH_2PO_4 was analytical grade supplied by Finerchem limited, Orthophosphoric acid (Merck), and Water and Methanol for HPLC (Lichrosolv (Merck).

Equipment and Chromatographic Conditions: The chromatography was performed on a Waters 2695 HPLC system, equipped with an auto sampler, UV detector and Empower 2 software. Analysis was carried out at 254 nm with column Agilent (C8), (150 X 4.6mm, 5µm), dimensions at 25^0C temperature. The optimized mobile phase consists of Buffer: Ammonium acetate: acetonitrile (60:40). Flow rate was maintained at 10µL.

Preparation of solutions:

Preparation of standard solutions

Accurately weighed and transferred about400mg of Gabapentine and 10mg of Nortriptyline into a 50ml volumetric flask, add about 30ml of diluents and sonicate for 30min with intermediate shaking (maintain the sonicator bath temperature between 20-25◦C). Make up to the volume with diluent and mix. Filter a portion of the solution through 0.45µm membrane filter and discard first few ml of the filtrate. Transfer 5 ml of the filtered solution into a 25ml volumetric flask, dilute to volume with diluent ad mix.

Preparation of sample solution

Commercially available 20 tablets are weighed and powdered equivalent to the 400mg of Gabapentine and 10mg of Nortriptyline into a 50ml volumetric flask, add about 30ml of diluents and sonicate for 30min with intermediate shaking (maintain the sonicator bath temperature between 20-25◦C). Make up to the volume with diluent and mix. Filter a portion of the solution through 0.45µm membrane filter and discard first few ml of the filtrate. Transfer 5 ml of the filtered solution into a 25ml volumetric flask, dilute to volume with diluent ad mix.

Preparation 0.1M Ammonium acetate buffer

Accurately weigh and transfer about 7.708g Ammonium acetate into a beaker containing 1000ml of water and sonicate to dissolve. Filter the solution through 0.45µm membrane filter.

Mobile phase preparation

The mobile phase used in this analysis consists of a mixture of Ammonium acetate and Acetronitrile in a ratio of 70:30. 700 ml of buffer was added and properly mixed with 300 ml of Ammonium acetate and a homogenous solution is achieved. This mobile phase was filled and sonicated for 15 minutes before using in the experiment.

Sample & standard preparation for the analysis

25 mg of Gabapentin and nortryptaline standard was transferred into 25 ml volumetric flask, dissolved & make up to volume with mobile phase.

Further dilution was done by transferring 0.1 ml of the above solution into a 10 ml volumetric flask and make up to volume with mobile phase.

The sample was analysed by HPLC by using the above method and a very nicely resolved peak has been obtained at a Retention Time of about 2.72 min. The respective chromatogram is attached in the following page.

RESULTS AND DISCUSSION:

METHOD:

The developed chromatographic method was validated for system suitability, linearity accuracy, precision, ruggedness and robustness as per ICH guidelines.

System suitability parameters: To evaluate system suitability parameters such as retention time, tailing factor and USP theoretical plate count, the mobile phase was allowed to flow through the column at a flow rate of 1.0 ml/min to equilibrate the column at ambient temperature. Chromatographic separation was achieved by injecting a volume of 10 µL of standard into Agilent (C8), (150 X 4.6mm, 5µm), the mobile phase of composition Buffer: Ammonium acetate: acetonitrile (60:40) was allowed to flow through the column at a flow rate of 1.0 ml per minute. Retention time, tailing factor and USP theoretical plate count of the developed method are shown in table 1.

Table 1: System suitability parameters

Parameter	Gabapentin	Nortriptyline
Theoretical plates	3234.04	3765.44
Retention time	2.66	3.682
Tailing factor	1.13	1.33

Assay of pharmaceutical formulation: The proposed validated method was successfully applied to determine Gabapentin and Nortriptyline in their tablet dosage form. The result obtained for was comparable with the corresponding labeled amounts and they were shown in Table-2.

Table 2: Assay results for Gabapentin and Nortriptyline

	Label Claim (mg)	% Assay
Gabapentin	400	100.15
Nortriptyline	10	100.30

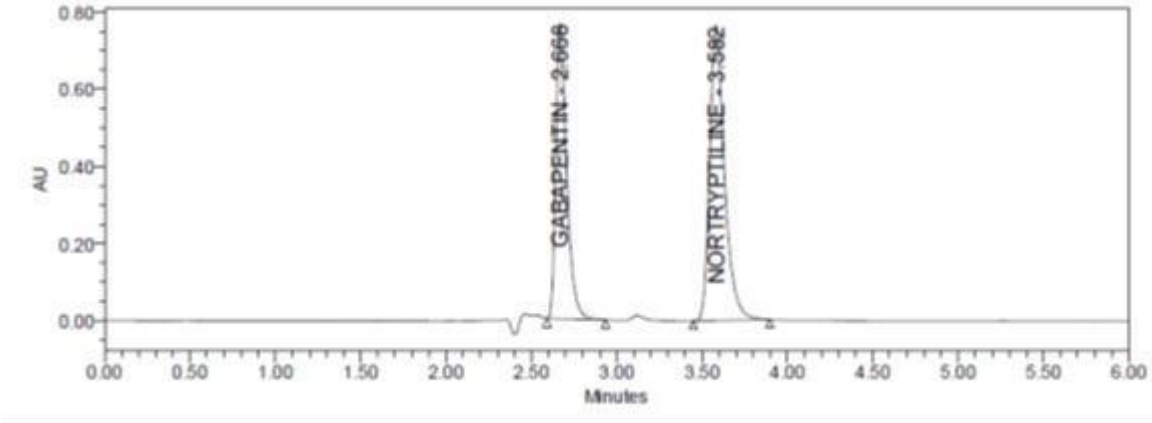

Figure 3: Standard chromatogram

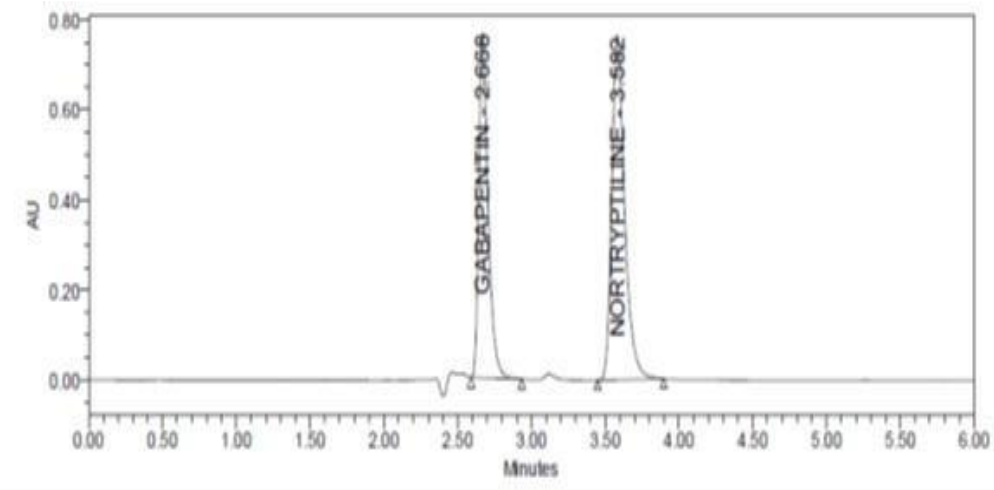

Figure 4: Sample chromatogram

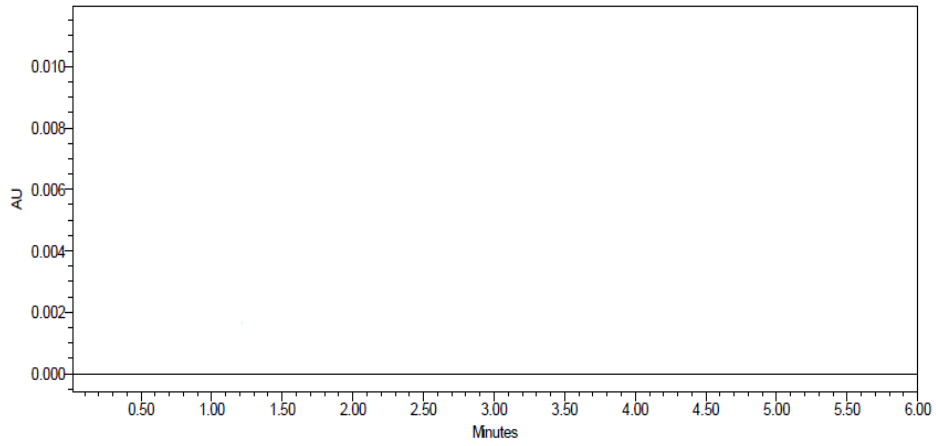

Figure 5: Blank chromatogram

Validation of Analytical method:

Linearity: Solutions were prepared containing 800 µg/ml, 1200 µg/ml, 1600 µg/ml, 2000 µg/ml, 2400 µg/ml, concentrations of Gabapentin and 20 µg/ml, 30 µg/ml, 40 µg/ml, 50 µg/ml, 60 µg/ml, concentrations of Nortriptyline which corresponding to 50, 75, 100, 125 and 150% respectively of the test solution concentration. Each solution was injected, linearity was evaluated by linear-regression analysis. The results are shown in table 3,4.

Table 3: Linearity results of Gabapentin

S. No:	**Concentration (µg/ml)**	**Peak Area**
1	20ppm	467525
2	30ppm	668668
3	40ppm	899412
4	50ppm	1128421
5	60ppm	1365426
6	70ppm	1594287
Mean		**1131243**
Co-relation Coefficient		**0.999**

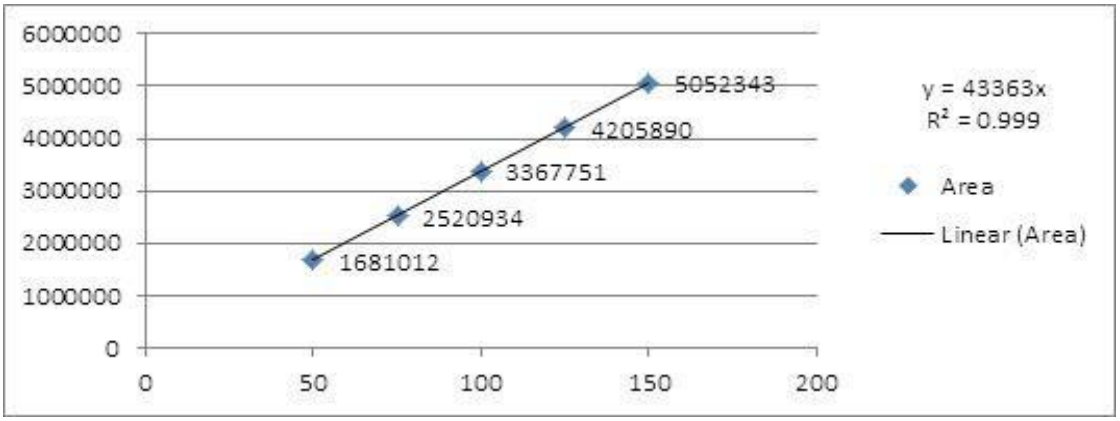

Figure 6: Linearity graph for Gabapentin

Table 4: Linearity results of Nortriptyline

S.No:	**Concentration (µg/ml)**	**Peak Area**
1	20ppm	467525
2	30ppm	668668
3	40ppm	899412
4	50ppm	1128421
5	60ppm	1365426
6	70ppm	1594287
Mean		**1131243**
Co-relation Coefficient		**0.999**

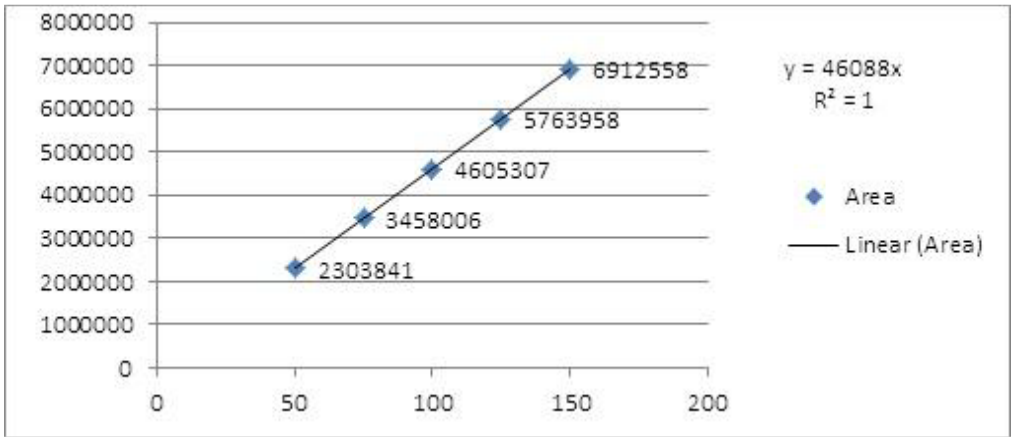

Figure 6: Linearity graph for Nortriptyline

Accuracy studies: Accuracy was determined by the recovery studies at three different concentrations (corresponding to 50, 100 and 150% of the test solution concentration) by addition of known amounts of standard to pre-analyzed sample preparation. For each concentration, three sets were prepared and injected. The results are shown in table 5.

Table 5: Showing accuracy results for Gabapentin and Nortriptyline

Sample ID	Concentration (µg/ml)		Rt	Peak Area	USP Plate Count	USP Tailing	% Recovery of	Statistical Analysis
	Amount Injected	Amount Found					Pure drug	
S_1 : 50 %	40	40.710	3.459	935684	3451	1.0	101.775	Mean= 101.440%
S_2 : 50 %	40	40.272	3.460	925689	3465	1.1	100.68	
S_3 : 50 %	40	40.747	3.461	936523	3448	1.0	101.867	% R.S.D.= 0.65098%
S_4 : 100 %	50	50.778	3.462	1165243	3964	1.4	101.556	Mean= 101.556%
S_5 : 100 %	50	50.784	3.461	1165382	3985	1.3	101.568	
S_6 : 100 %	50	50.772	3.459	1165121	3958	1.4	101.544	%R.S.D.= 0.01181%
S_7 : 150 %	60	59.559	3.464	1365482	3797	1.6	99.265	Mean= 100.243%
S_8 : 150 %	60	60.436	3.463	1385462	3746	1.6	100.726	
S_9 : 150 %	60	60.443	3.464	1385643	3789	1.6	100.738	%R.S.D. = 0.84494%

Precision Studies: Intraday variations were determined by using six replicate injections of one concentration and analyzed on the same day and different days. Precision of an analytical method is usually expressed as the standard deviation or relative standard deviation (coefficient of variation) of a series of measurements. The results are shown in table 6.

Table 6: Precision results for Gabapentin and Nortriptyline

HPLC Injection Replicates of Gabapentine and Nortryptaline	Rt	Peak Area	USP Plate Count	USP Tailing
Replicate – 1	3.461	1065243	3986	1.5
Replicate – 2	3.460	1056842	3956	1.4
Replicate – 3	3.459	1065341	3987	1.5
Replicate – 4	3.461	1064512	3926	1.4
Replicate – 5	3.460	1056864	3963	1.5
Replicate – 6	3.459	1056845	3951	1.4
Mean	**3.46**	**1060941**	**3961.5**	**1.45**
Standard Deviation	**0.000894**	**4490.439**		
% RSD	**0.02585**	**0.42325**		

Ruggedness: To evaluate the intermediate precision of the method, Precision was performed on different day. The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found. The results are shown in table 7.

Table 7: Ruggedness results of Gabapentin and Nortriptyline

S.No	Sample Weight	GPT sample area	NTL Sample area	GPT % Assay	NTL % Assay
1	765.55	3364530	4601901	99.01	99.68
2	765.55	3364541	4606209	99.01	99.77
3	765.55	3365736	4603520	99.04	99.71
4	765.55	3371195	4614415	99.20	99.95
5	765.55	3371160	4609760	99.20	99.85
6	765.55	3370202	4614992	99.17	99.96
Avg Assay:				99.11	99.82
STD				0.10	0.12
% RSD				0.10	0.12

Robustness: The robustness was evaluated by assaying test solutions after slight but deliberate changes in the analytical conditions. The factors chosen for this study were the flow rate (±0.1ml/min) and temperature (±5 0C). The results are shown in table 8.

Table 8: Robustness results of Gabapentin and Nortriptyline

S.No	Parameter changing	Area	USP Tailing	USP Plate count	Rt	
					GPT	NTL
1.	Temp1	4686059	1.36	8709	3.59	---
2.	Temp2	4691248	1.36	8734	3.59	---
3.	Flow1	6073408	1.37	9828	4.48	---
4.	Flow2	3883341	1.33	8081	2.99	---
5.	Temp1	3236283	1.51	8648	---	2.67
6.	Temp2	3278704	1.49	8690	---	2.67
7.	Flow1	4162866	1.55	9585	---	3.35
8.	Flow2	2683053	1.48	8363	---	2.22

LOD and LOQ: The sensitivity of RP-HPLC was determined from LOD and LOQ. Which were calculated from the calibration curve using the following equations as per ICH guidelines. The results are shown in table 9.

LOD = 3.3σ/S and

LOQ = 10 σ/S, where

σ= Standard deviation of y intercept of regression line,

S = Slope of the calibration curve

Table 9: LOD, LOQ of Gabapentin and Nortriptyline

Drug	LOD	LOQ
Gabapentin	2.936	9.786
Nortriptyline	2.927	9.756

CONCLUSION:

The Developed HPLC method was validated and it was found to be simple, precise, accurate and sensitive for the simultaneous estimation of Gabapentin and Nortriptyline in its bulk and tablet dosage form. Hence, this method can easily and

conveniently adopt for routine quality control analysis of Nortriptyline and Gabapentin in its bulk and tablet dosage form.

REFERENCES:

1. FDA Approved Drug Products: Neurontin (gabapentin) for oral use
2. EMA Approved Drugs: Gabapentin
3. Sills GJ: The mechanisms of action of gabapentin and pregabalin. Curr Opin Pharmacol. 2006 Feb;6(1):108-13. doi: 10.1016/j.coph.2005.11.003. Epub 2005 Dec 22. (PubMed ID 16376147)
4. Maneuf YP, Luo ZD, Lee K: alpha2delta and the mechanism of action of gabapentin in the treatment of pain. Semin Cell Dev Biol. 2006 Oct;17(5):565-70. Epub 2006 Sep 24. (PubMed ID 17067834)
5. Kukkar A, Bali A, Singh N, Jaggi AS: Implications and mechanism of action of gabapentin in neuropathic pain. Arch Pharm Res. 2013 Mar;36(3):237-51. doi: 10.1007/s12272-013-0057-y. Epub 2013 Feb 24. (PubMed ID 23435945)
6. FDA Approved Products: Nortriptyline oral capsules and oral solution
7. Clouse RE: Antidepressants for irritable bowel syndrome. Gut. 2003 Apr;52(4):598-9. doi: 10.1136/gut.52.4.598. (PubMed ID 12631677)
8. Rang, H. P. and Dale, M. M. (2012). Rang and Dale's Pharmacology (7th ed.). Edinburgh: Elsevier/Churchill Livingstone. (ISBN 978-0-7020-3471-8)
9. Cusack B, Nelson A, Richelson E: Binding of antidepressants to human brain receptors: focus on newer generation compounds. Psychopharmacology (Berl). 1994 May;114(4):559-65. (PubMed ID 7855217)
10. Shaikh, Sultana & Jain, Vandana. (2018). Development and validation of novel RP-HPLC method for simultaneous estimation of gabapentin and amitriptyline hydrochloride in bulk and pharmaceutical dosage forms. International Journal of Applied Pharmaceutics. 10. 97. 10.22159/ijap.2018v10i2.24842.
11. Patel Y., Patel M. B, Patel N.K., Sakhreliya B. Development and Validation of Analytical Method for Simultaneous estimation of Gabapentin and Nortriptyline Hydrochloride in Pharmaceutical Dosage Form. J Pharm Sci Bioscientific Res. 2015 5(5):443-443
12. Gadarajan TVS, Arunkumar N, Aiyalu R, Ponnilavarasan I, Tamilselvi N. Development and Validation of RP-HPLC Method for the Simultaneous Estimation of Gabapentin and Nortriptyline Hydrochloride in Bulk and Tablet Dosage Form. J Young Pharm. 2022;14(2):187-91.