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DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF GABAPENTIN AND NORTRYPTILINE IN PHARMACEUTICAL DOSAGE FORM

M.NaveenKumar*, D. SaiMalakondaiah, G.Usha Sree, A.Ajitha, V.Uma Maheshwara Rao

Department of Pharmaceutical Analysis and Quality Assurance, CMR College of Pharmacy,
Kandlakoya (v), Medchal road, Hyderabad – 501 401, A.P, 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 80:20v/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.

INTRODUCTION

Gabapentin (GPT) is an antilipemic agent and chemical it is 2-[1-(aminomethyl)cyclohexyl]acetic acid (MolWt- 171.23), Gabapentin increases the synaptic concentration of GABA, enhances GABA responses at non-synaptic sites in neuronal tissues, and reduces the release of mono-amine neurotransmitters and it is used Anti- epilepsy, Anti-anxiety Agent, Antiparkinson Agents [1].

Nortriptyline(NTL) is an Antidepressant and chemically it is 3-(10,11-Dihydro- 5*H*- dibenzo [a,d] cyclohepten-5-ylidene)-*N*-methyl-1-propanamine (Mol Wt-263.38). It inhibits the reuptake of the neurotransmitter serotonin at the neuronal membrane or acts at beta-adrenergic receptors It is used as an Antidepressant, Norepinephrine-Reuptake Inhibitors [2].

From the literature review it is found that there is no official RP-HPLC method in Pharmacopoeia for the simultaneous estimation of Gabapentin and Nortriptyline in pharmaceutical tablet dosage form [3-10]. A very few analytical methods are available for estimation of Gabapentin alone and its combination with drugs such as UV and HPLC [11-15]. The combination therapy is used for Neuropathy. In monotherapy, the maximum tolerated doses, hardly reduce pain by not more than 60 percent. In addition, they provide relief in only 40 to 60 percent of patients because of partial efficacy and dose-limiting side-effect [16]. The combination of nortriptyline and gabapentin decreases pain more than either medication alone in the treatment of neuropathic pain from diabetic polyneuropathy or postherpetic neuralgia. Hence here is an

Corresponding Author:-M.NaveenKumar Email:- Mallepallynaveenkumar@gmail.com

attempt to develop a more precise and accurate HPLC method for the simultaneous determination Gabapentin and Nortrptyline of in pharmaceutical solid dosage forms. Various validation aspects of the analysis accuracy, precision, recovery, the limits of detection and quantification etc have been measured as per ICH guidelines [17].

MATERIALSANDMETHODS

Equipment

Chromatographic separation was performed on HPLC system-Water's 2690 series, PDA Detector 2695 series, equipped with a solvent delivery pump, sample injector and column thermostats. Empower software was applied for data collecting and processing.

Chemicals andreagents

Methanol, Water of HPLC grade was purchased from Merck Chemicals (Mumbai, India).Buffer: Ammonium acetate (0.1M) used was from LOBA Chemie Pvt). Reference standards Gabapentin and Nortrptyline were obtained from Rainbow labs. Gabapin NT, Tablets with Gabapentin (400 mg) & Nortriptyline (10 mg), manufactured by INTAS pharmaceuticals Limited, were procured from local market.

Preparation ofstandardsolutions

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 samplesolution

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.

Optimizedchromatographicconditions

Diluent : Methanol
Mobile phase:Buffer:Ammonium acetate:Methanol (60:40)
Flow rate : 1.0 mL/min
Column :Agilent (C8), (150 X 4.6mm, 5µm)
Detector wavelength : 254nm
Injection volume : 10µL
Run time : 6 min
Mode of Pump : Isocratic

METHODVALIDATION

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.

Accuracy

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-analysed sample preparation. For each concentration, three sets were prepared and injected.

Precision

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.

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 ^{0}C).

Limit of detection (LOD) and Limit of quantification (LOQ)

LOD and LOQ was calculated from linear curve using formulae LOD=3.3*σ /slope, LOQ=10*σ /slope
(Where σ = the standard deviation of the response and,
S= Slope of calibration curve).

The LOD was 2.936 and 2.927 µg mL-1 for GPT and NTL respectively and, the respective LOQ were 9.786 and 9.756 µg mL-1.

Specificity

Specificity was checked for the interference of impurities in the analysis of blank solution and injecting sample solution under optimized chromatographic conditions to demonstrate separation of both Gabapentin and Nortriptyline from impurities.

Table1. Result of Linearity

Linearity conc (%)	Final Conc (µg/mL)		Peak Area		LOD (µg/ml)	LOQ (µg/ml)
	GPT	NTL	GPT	NTL	GPT	
50	800	20	1681012	2303841	2.936	9.786
75	1200.00	30	2520934	3458006		
100	1600.00	40	3367751	4605307	NTL	
125	2000	50	4205890	5763958	2.9268	9.7561
150	2400	60.00	5052343	6912558		

Table 2. System suitability studies

Parameters	GPT	NTL	Acceptance criteria
Theoretical plates	8734	8648	Not less than 2000
Tailing factor	1.52	1.37	Not more than 2
Resolution	-	6.56	Not less than 2

Table 3. Recovery studies for Gabapentine and Nortriptyline

Drug	Spiked level%	Amount taken (µg/ml)	Amount found (µg/ml)	Percent recovery n=3	Mean recovery
GPT	50	792.810	793.627	100.10	100.01
	100	1585.600	1581.546	99.74	
	150	2378.348	2376.828	99.94	
NTL	50	19.940	19.948	100.04	99.94
	100	39.880	39.888	100.02	
	150	59.819	59.810	99.99	

n- Number of replicate injections

Table 4.ResultsofIntraday Precision

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

Table 5. Results of Robustness study

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

Figure 1. Chromatogram of GPT (1600 µg/mL) and NTL (40µg/mL) standard

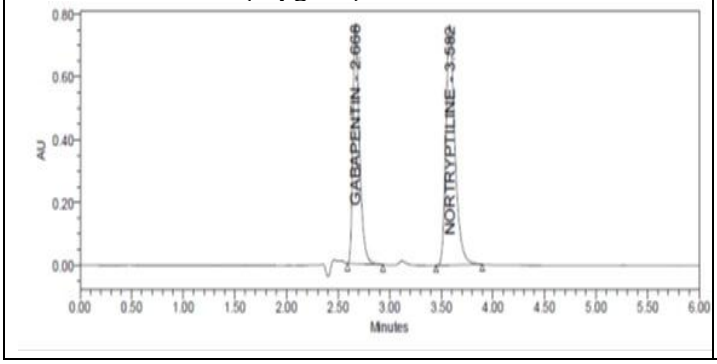


Figure 2. Chromatogram of GPT (1600µg/mL) and NTL (40µg/mL) sample

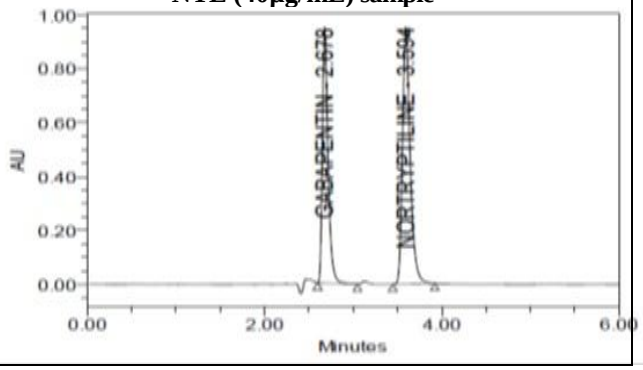


Figure 3. Calibration curve for Gabapentine

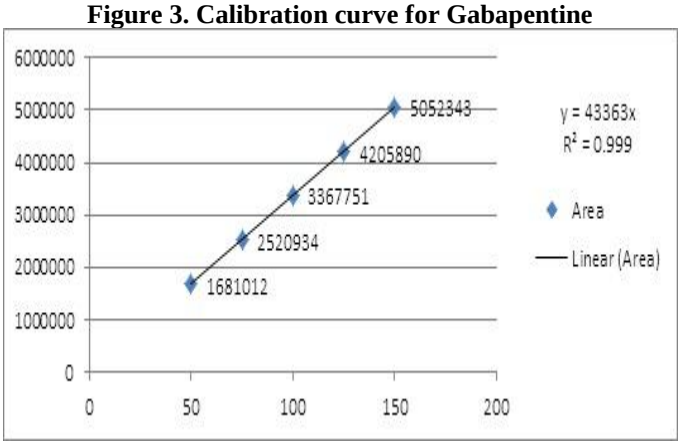
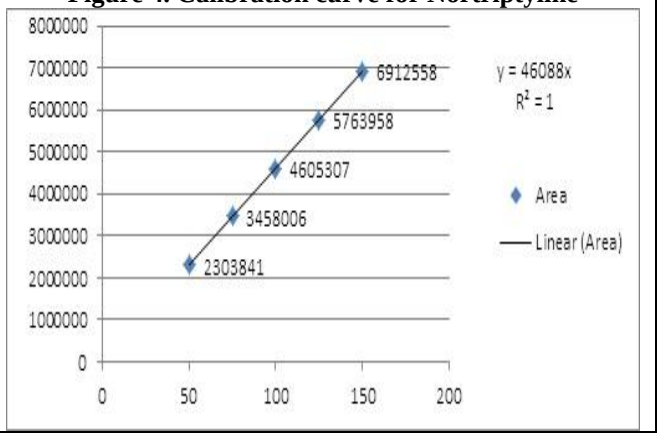


Figure 4. Calibration curve for Nortriptyline



RESULTS AND DISCUSSION

Several mobile phase compositions were tried to resolve the peak of GPT and NTL. The mobile phase containing buffer: Ammonium acetate (0.1M): Methanol in proportion of 80:20 v/v was found ideal to resolve the peak of GPT and NTL. Retention time of GPT and NTL were 2.66 and 3.58 min respectively (Figure 1&2). Result of assay is shown in Table-3. The proposed method was found to be linear in concentration range 800-2400µg/ml for GPT and 20-60 µg/ml for NTL. The data was shown in Table-1 and Figure 3 & 4. System suitability parameters were evaluated and results shown in (Table-2), which were within acceptance criteria. The mean percentage recovery for GPT and NTL was found to be 99.18% and 99.18% respectively, which are well within the limit and hence the method was found to be accurate (Table-3). LOD and LOQ values were 2.936 µg/mL and 9.786 µg/mL for GPT and 2.927 µg/mL and 9.756 µg/mL for NTL (Table 1). Results of intraday precision were shownin (Table-4). The robustness of the method was investigated by varying experimental conditions such as changes in flow rate and column temperature. The result obtained implies method is robust for routine qualitative analysis (Table 5).

CONCLUSION

Theproposed RP-HPLC methodwasvalidated asper InternationalConferenceonHarmonization (ICH) guidelines and found to be applicable for routine quality control analysis for the simultaneous estimation of GPT and NTL using isocratic mode of elution. The results of linearity, precision, accuracy and specificity, proved to be within the limits. The proposed method is highly sensitive, reproducible, reliable, rapid and specific.

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