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Original Article

Development and Validation of RP-HPLC Method for the Simultaneous Estimation of Gabapentin and Nortriptyline Hydrochloride in Bulk and Tablet Dosage Form

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ABSTRACT

Background: According to ICH requirements, a simple isocratic RP-HPLC technique for the simultaneous estimation of gabapentin (GAB) and nortriptyline HCl (NOR) in pharmaceutical dosage forms has been developed and validated. **Methods:** On a reversed phase, separation is accomplished Phenomenex Luna C_{18} Column (5µm, 250 × 4.60 mm) as a stationary phase using two solvents (Solvent A: buffer 0.2 % Triethylamine adjusted to pH 5.5 with orthophosphoric acid and Solvent B: Acetonitrile) as a mobile phase. The ratio of Solvent A: Solvent B was 50:50 v/v. The flow rate was 1.2 ml/min with UV detection at 210 nm. **Results:** The retention time for Gabapentin and Nortriptyline HCl was found to be 1.96 and 4.54 min respectively with runtime of 15 min, theoretical plate for GAB and NOR were 3936 and 3912 respectively, with a resolution of 8.5. Linearity of GAB and NOR was found in the range of 200-1000 µg/ml and 5-25 µg/ml. The correlation coefficient for GAB and NOR were 0.9995 and 0.9998. The LOD values for GAB and NOR were 62.729 and 0.8727 µg/ml. The LOQ values for GAB and NOR were 190.09 and 2.6447 µg/ml respectively. The percentage of GAB and NOR in pharmaceutical dosage form was found to be 99.79 % and 99.75 %. **Conclusion:** This demonstrates that the developed method is simple, precise, rapid, selective, accurate and reproducible for simultaneous estimation of GAB and NOR tablet dosage form.

Key words: Dosage formulation, Gabapentin, Method validation, RP-HPLC, Nortriptyline HCl UV detection.

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INTRODUCTION

Gabapentin (GAB) is an antiepileptic agent and chemically it is known as 2- [1-(amino methyl) cyclohexyl] acetic acid.[1] It is used as anti-epileptic, anti-anxiety agent, antiparkinson agents.[2] Nortriptyline hydrochloride (NOR) is an antidepressant drug and chemically it is called as 3-(10,11-dihydro- 5*H*- dibenzo [a,d] cyclohepten-5-ylidene)-*N*-methyl-1-propanamine.[3] In this combination the Gabapentin used as a anticonvulsant to treat the convulsion which cause depression as a side effect. So, to treat the depression Nortriptyline hydrochloride used as antidepressant to treat the depression. The combined dosage form of Gabapentin and Nortriptyline HCl has recently been introduced in the market, where the co administration of Gabapentin and Nortriptyline HCl offers a well-tolerated and highly efficient new treatment option for patients with convulsion.[4]

MATERIALS AND METHODS

Drug and chemicals

Gabapentin and Nortriptyline hydrochloride Working Standard procured as gift sample by Synthia Research Lab Pvt Ltd, Pondicherry, India. Gabapentin 400mg and Nortriptyline hydrochloride 10mg (GABARIDE-NT) procured in pharmacy outlet, manufactured by Medopharm. The HPLC grade acetonitrile Merck. Triethylamine and orthophosphoric acid were of Analytical grade. HPLC grade water Millipore MilliQ plus water purification system.

Instruments used

Elico pH meter LI 127, Shimadzu LC-20 AT HPLC, Shimadzu 1800 UV Spectrophotometer, Sonica Ultrasonic cleaner, solvent filtration unit – Millipore, Shimadzu electronic balance AX 200.

Preparation of Standard solutions

In a 50 ml volumetric flask, accurately weigh and transfer around 100 mg Gabapentin and 2.5 mg Nortriptyline Hydrochloride, add about 30 ml diluents, and sonicate for 30 min with intermediate shaking (keep the sonicator bath temperature between 20°C-25°C). Add diluent to make up the volume, then mix. Filter a portion of the solution through 0.45 µm membrane filter and discard first few ml of the filtrate. Transfer 2 ml of the filtered solution into a 10 ml volumetric flask, dilute to volume with diluent ad mix to get final concentration of 400 µg/ml and 10 µg/ml for Gabapentin and Nortriptyline Hydrochloride respectively.

Preparation of sample solution

In a 50ml volumetric flask, weigh and powder 20 commercially available tablets equivalent to 100 mg Gabapentin and 2.5 mg Nortriptyline, add 30ml diluents, and sonicate for 30 min with intermediate shaking (keep the sonicator bath temperature between 20°C-25°C). Make up to the volume with diluent and mix. Filter a portion of the solution through 0.45 µm membrane filter and discard first few ml of the filtrate. Fill a 10 ml volumetric flask with 2 ml of the filtered solution, dilute to volume with diluent add mix to get final concentration of 400 µg/ml and 10 µg/ml for Gabapentin and Nortriptyline Hydrochloride respectively.

RESULTS

Method development

Optimized Chromatographic condition

Shimadzu HPLC with LC-20AT prominence liquid chromatogram, Rheodyne 7725i with 20 µl loop injector, SPD-M20A Prominence-diode

array detector and Sonica ultrasonic cleaner sonicator was used. lab solution software recorded and processed the chromatograms. Finally, using 0.2 % triethylamine pH 5.5(adjusted with OPA) and acetonitrile in a 50:50 percent v/v ratio at a flow rate of 1.2 ml/min, a phenomenonx luna C_{18}(250 x 4.6 mm 5 m) column as the stationary phase, and the eluents monitored at a wavelength of 210 nm, A symmetric analyte peak was obtained with an appropriate short run time. The retention time for Gabapentin and Nortriptyline HCl was found to be 1.96 and 4.54 min respectively (Figure 1).

Method validation

The developed RP-HPLC technique was designed to measure Gabapentin and Nortriptyline HCl. accuracy, precision, linearity, specificity, robustness, detection limit, and quantitative limit of the developed technique were all validated in compliance with the ICH guidelines.[5-9]

Linearity and range

The calibration curve was plotted against the matching peak area for five distinct drug concentrations. Within the concentration ranges of 200-100 g/ml for Gabapentin (Figure 2) and 5-25 g/ml for Nortriptyline HCl (Figure 3), there was an excellent connection between concentrations and peak area (Figure 3). Gabapentin having a correlation coefficient of 0.9995 and Nortriptyline HCl having a correlation coefficient of 0.9998.

Accuracy (% Recovery)

Recovery experiments were used to assess the method's accuracy. A known quantity of the pure drug sample was added to the pre-analyzed same formulation at 50 %, 100 % and 150 % levels. The percentage recovery for Gabapentin and Nortriptyline HCl was determined to be 99.98%, 99.87 %, 99.99 % and 99.93 %, 99.45 %, 99.96 % respectively. Gabapentin had a percentage relative standard deviation of 0.23 % and Nortriptyline HCl had a percentage relative standard deviation of 0.35 %. The assay mean recovery is within the prescribed limits, and the percent RSD is less than 1.0 percent, according to the accuracy results. The validation criterion was enumerated in (Table 1).

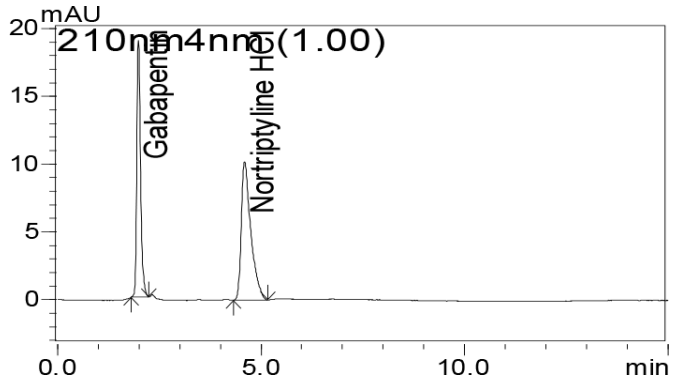

Figure 1: Chromatogram of Gabapentin and Nortriptyline HCl.

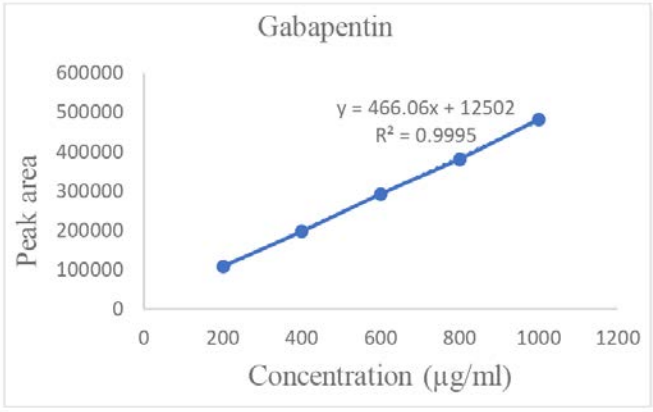

Figure 2: Linearity graph for Gabapentin.

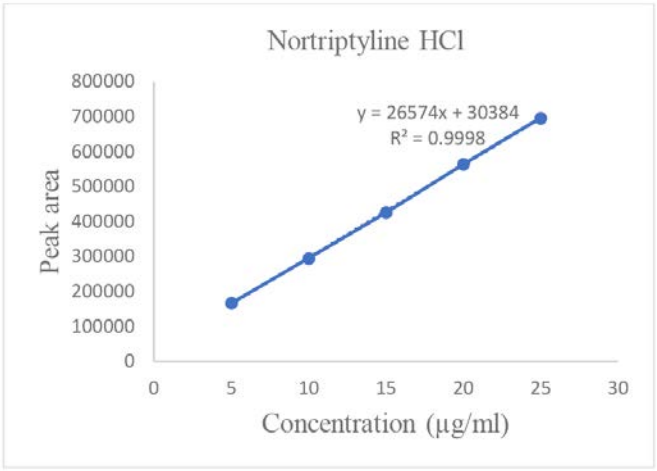

Figure 3: Linearity graph for Nortriptyline HCl.

Precision

In this method was determined by studying repeatability and reproducibility. The RSD of the peak areas of six replicates was found 0.32 for GAB and 0.2 for NOR in intraday studies. Interday trials were also conducted, and RSD was determined to be 0.3 for GAB and 0.25 for NOR (Table 2). The percent RSD values were determined to be less than 1%, indicating that the approach is accurate.

Specificity

The specificity of a technique relates to its ability to measure analyte response in the presence of additional excipients and contaminants. By comparing the chromatogram of the medication extracted from the tablet to that of the reference solution for excipients, potential impurities, and other degradants, the specificity was established. There was no interference in the sample solution during the retention period of Gabapentin and Nortriptyline HCl.

Detection limit and Quantitation limit

The suggested method's sensitivity is represented by the Detection and Quantification limit. The LOD and LOQ for GAB were determined to be 62.729 and 190.09 g/ml, respectively, and 0.8727 and 2.6447 g/ml for NOR, showing the method's sensitivity.

Robustness

The robustness of an analytical technique is a measure of its ability to stay unaffected by modest but purposeful changes in method parameters, and it indicates the method's dependability in routine use. The present technique's robustness was examined by evaluating samples of the drug product under the same chromatographic circumstances as in method development, but with a moderate variation in the following chromatographic parameters, such as changing the composition of the mobile phase (±2), pH 5.5 (± 0.1) %) and flow rate 1.2 ml/min(±0.1 ml/min),. The results are reported in (Table 3).

Table 1: Accuracy studies of Gabapentin and Nortriptyline HCl.

Drug	Label claim mg/tab	Spike Level (%)	Amount of drug added (µg/ml)	Amount of API added (µg/ml)	Amount of drug recovered (µg/ml)	Percentage Recovery (%)	%RSD*
GAB	400	50	200	400	599.89	99.98	0.39
		100	400	400	798.99	99.87	0.23
		150	600	400	999.98	99.99	0.32
NOR	10	50	5	10	14.99	99.93	0.22
		100	10	10	19.89	99.45	0.35
		150	15	10	24.99	99.96	0.33

*-Each value is the mean of six observations.

Table 2: Intraday and Interday precision characteristics of Gabapentin and Nortriptyline HCl.

Parameters	Peak area Gabapentin	Peak area Nortriptyline HCl
Intraday	292336	425927
	292213	424361
	292453	424323
	291327	425842
	294351	425761
	293214	426574
Average*	292649	425464
%RSD	0.32	0.2
Day 1	293214	426574
Day 2	294351	425761
Day3	292312	424321
Day 4	293832	424355
Day 5	292352	423452
Day 6	294532	423452
Average*	293432	425025
%RSD	0.3	0.25

*-Each value is the mean of six observations

System suitability

System suitability factors are an important aspect in developing and validating analytical methods. They ensure the system's best performance. Parameters for chromatography Viz., Theoretical plate count (N), retention time (R*t*), resolution (Rs), and peak asymmetry factor (A) After six duplicate injections of conventional Gabapentin and Nortriptyline HCl at concentrations of 600 g/ml and 15 g/ml, respectively, were measured. The data is shown in (Table 4).

Assay of Marketed Tablet dosage form

The proportion of GAB and NOR (Gabaride-NT) formulation was determined to be 99.79 percent and 99.75 percent, respectively, in an assay of Pharmaceutical tablet dosage form utilising the established and validated technique. The approach was shown to be very precise, since the produced peaks did not interact with the standard peaks in any way. The approach was confirmed to be reliable because the calculated percent RSD values were less than 0.06 percent.

Table 3: Robustness.

Chromatographic Condition	Retention time for GAB	Retention time for NOR
Flow rate (ml/min)		
1.1	2.156	5.008
1.2	1.973	4.563
1.3	1.832	4.276
pH of Mobile phase		
5.4	1.921	4.276
5.5	1.972	4.521
5.6	1.832	4.753
% Acetonitrile Concentration		
52	1.967	4.261
50	1.973	4.563
48	1.984	5.079

Table 4: System suitability parameters.

Parameters	Gabapentin	Nortriptyline HCl
Retention time	1.967	4.549
Peak purity index	0.9975	0.9877
Resolution factor (Rs)	8.5	8.5
No. of theoretical plates (N)	3936.5	3912.9
Height equivalent to theoretical plates (HETP)	77.45	78.41
Tailing factor	1.472	1.833

DISCUSSION

According to the literature review, no official method for measurement of combined tablet dosage form exists in the Pharmacopoeias. Only a few analytical procedures, such as UV and HPLC are available to calculate Gabapentin alone or in conjunction with other medications.[10,11] So far, there have only been three techniques developed and verified. In the first developed method, Luna C_{18} column used with mobile phase composition of water: Acetonitrile in 60:40 v/v ratio with a flow rate 1.2 ml/min. GAB and NOR had R*t* of 3.457 and 4.728 min, respectively.[12] In the second method, separation performed on Agilent C_8 column with mobile phase comprising of mixture of buffer: (0.1 M ammonium acetate) and Methanol (80:20 v/v) at the flow rate 1.0 ml/min. The retention times

were 2.66 and 3.58 min, respectively.[13] In the third method, flow rate 1.0 ml/min and C_8 column (150×4.6 mm,5 µm), The mobile phase ratio of Methanol: Acetonitrile: 0.028 M phosphate Buffer water (35: 40: 25 v/ v/v) pH 4.5 with O- Phosphoric acid was used and The retention times were 1.61 and 2.6 min, respectively.[14] When compared to the above three methods, this method Gabapentin and Nortriptyline HCl have retention time 1.967 and 4.549 min which is less when compared to those three methods and a good separation is achieved. The Phenomenex Luna C_{18} Column (250 × 4.60 mm, 5µm) was used as a stationary phase using two solvents (Solvent A: buffer 0.2 % Triethylamine adjusted to pH 5.5 with orthophosphoric acid and Solvent B: Acetonitrile mobile phase in the ratio (50:50 v/v). When compared to the other three techniques, the mobile phase was the most cost-effective and accessible. The C_{18} column which we used is the most preferred as they offer an excellent range of hydrophobic separation power along with high surface area coverage.[15] The devised technique may be utilised to quantify Gabapentin and Nortriptyline HCl in a short time while maintaining a high level of linearity. The various validation aspects of the analytical method such as accuracy, precision, linearity recovery, the limits of detection, limits of quantification, robustness, system suitability have been measured as per ICH guidelines.[16] The suggested method's analytical conditions were chosen based on the chemical nature of nortriptyline and gabapentin. Gabapentin and Nortriptyline HCl have maximum UV absorbance (max) at 210 nm and 221 nm, respectively, according to preliminary spectroscopic research. As a result, chromatographic detection was carried out at 210 nm with a photo diode array detector since both chemicals responded well at this wavelength. Each component was put through its paces in various harsh environments throughout development. Back pressure, resolution, peak form, theoretical plate, day-to-day repeatability of the retention duration, and resolution between nortriptyline and gabapentin peak were all factors in column selection. Phenomenex luna C_{18} column was chosen after considering all of these variables. It was deemed to be appropriate since it produced good outcomes. The buffer is chosen depending on the chemical composition of the medicines. Both components' solubility, resolution, stability, theoretical plates, and peak shape were determined to be best in the acidic pH range. For Gabapentin and Nortriptyline HCl, the best results were achieved with a 0.2 percent Triethylamine buffer (5.5). To lower the longer retention time and achieve appropriate peak shape, acetonitrile was selected as an organic ingredient of the mobile phase. For Gabapentin and Nortriptyline HCl, preliminary studies utilising alternative mobile phase compositions of buffer (4.5) and acetonitrile in the ratios of 60:40 v/v and 40:60 v/v did not yield excellent peak shape. Finally, the separation and resolution of nortriptyline and gabapentin are optimized. simply adjusting the mobile phase composition, which is a 50:50 v/v combination of buffer and acetonitrile. Gabapentin and Nortriptyline HCl eluted at 1.967 and 4.549 min, respectively, with a 15-min run duration under these circumstances. The flow rate was kept at 1.2 ml/min, and the eluents were monitored at 210 nm, and the mobile phase percentage was optimized to offer high resolution between gabapentin and nortriptyline HCl.

CONCLUSION

The validation of a rapid, simple, sensitive, precise, and accurate RP-HPLC technique was carried out according to ICH guidelines. The procedure accuracy was indicated by the relative standard deviation of 0.2 percent. In the concentration ranges of 200 µg/ml -1000 µg/ml and 5 µg/ml -25 µg/ml, respectively, gabapentin and nortriptyline HCl exhibited good linearity. The method's accuracy was demonstrated in recovery tests. The current verified method's mean recovery varied from 99 to 100 percent. As a result, an accurate, precise, and rapid RP HPLC technique for the routine quantification of Gabapentin and Nortriptyline HCl in bulk and pharmaceutical formulations was developed and validated.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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