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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF GABAPENTIN AND NORTRIPTYLINE HYDROCHLORIDE IN BULK AND COMBINED DOSAGE FORM

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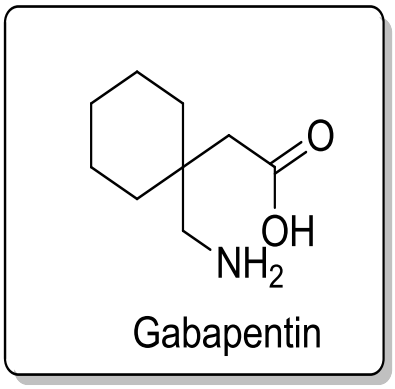
ABSTRACT

A new Simple, Precise, Fast and Accurate RP-HPLC Method has been developed and validated for simultaneous estimation of Gabapentin (GABAP) and Nortriptyline Hydrochloride (NTH) in bulk and combined dosage form. The method was carried out on the chromatographic separation was achieved by isocratic elution technique on (reverse phase) Luna C_{18} column (250 mm x 4.6 mm ID, Particle size 5µm), using mobile phase composition of Water: acetonitrile in the ratio of 60:40 v/v with a flow rate 1.2ml/min. Quantization was achieved with PDA detector 255nm based on peak area. The retention time of GABAP and NTH were found to be 3.457min and 4.728min respectively. The GABAP and NTH followed linearity in the concentration range of 50-500µg/ml and 1-12.5µg/ml respectively with r^2=0.999 for both GABAP and NTH .The amount of both drugs estimated by the proposed method was found to be in good agreement with labelled claim. The developed method was validated for precision, accuracy, sensitivity, robustness and ruggedness. The developed method can be used for routine analysis of titled drugs in combined dosage form.

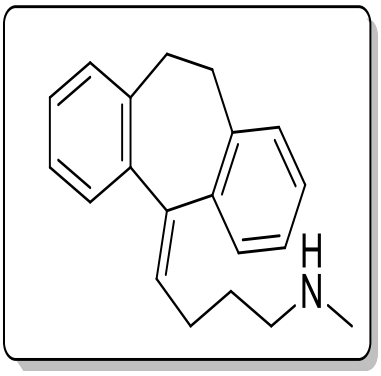
KEYWORDS: Gabapentin and Nortriptyline hydrochloride, RP-HPLC validation C_{18} Column ICH Guidelines.

INTRODUCTION

Gabapentin (GBP) 2-[1-(amino methyl) cyclohexyl] acetic acid (Anonymous 1), is an antiepileptic drug which is a structural analogue of neurotransmitter γ-amino butyric acid (GABA). It is orally administered and its bio-availability is rapid, in part by saturable carrier-mediated L-amino acid transport system. It has less protein binding (<3%) and half-life is about 5ll/-7 hours. Freely soluble in water and alkaline and acidic solutions. Nortriptyline hydrochloride, 3-(10,11-dihydro-5H-dibenzo [a, d]cyclohept- 5-ylidene) N-methyl propylamine hydrochloride, is a tri-cyclic anti-depressant and fluphenazine hydrochloride, 2,4-[3-(2-trifluoro-methyl phenothiazine-10-yl) propyl] piperazine-1-yl-ethanol dihydrochloride, is a tricyclicneuroleptic agent. moderately soluble in water at room temperature but very soluble in Boiling water It is also moderately soluble in ethanol.



Gabapentin



MATERIALS AND METHODS

Chemicals

Gabapentin, NortriptylineHydrochloride was purchased from Indian market manufactured by Ranbaxy, Hyderabad. Commercial pharmaceutical preparation of Gabapentin, Nortriptyline Hydrochloride tablets which are claimed to contains 400mg; 10mg of (Gabacent NT) were used in analysis. HPLC grade water from I-con laboratories.

Instrumentation

Analysis was performed on waters HPLC, 2695 Empower software used separation module equipped with PDA detector, Auto sampler and column compartment with Empower 2 software. Other equipment used in the study was analytical balance (DENVER) and P^H meter (EUTECH instrument). Ultra sonic bath (UNICROME ASSOCIATES: UCA-701).

Chromatographic Conditions

Luna C_{18} column (250 mm x 4.6 mm ID, Particle size 5µm) was used for chromatographic separation using mobile phase composition of Water: acetonitrile in the ratio of 60:40 v/v with a flow rate 1.2ml/min. with run time 8 min Mobile phase and sample solutions were filtered through a 0.45 µm membrane filter and degassed. The detection of both drugs was carried out at 255 nm.

METHOD DEVELOPMENT

Standard stock solutions of 10mg/ml of Gabapentin and Nortriptyline Hydrochloride were prepared separately using diluent (Buffer: Acetonitrile - 45:55v/v). The GABP stock solution was diluted with diluent to give working standard solution containing 50-500 µg/ml concentration. Similarly the NTH stock solution was diluted with diluent to give working standard solution in the range 1-12.5 µg/ml. These solutions were filled into vials and placed in vial holder. The linearity was determined separately for GABP and NTH by injecting eight concentrations of both drugs prepared in diluent and calibration curves were constructed by plotting area against the respective concentrations

VALIDATION OF METHOD

The HPLC method was validated in accordance with ICH guidelines. The system precision of the method was verified by six replicate injections of standard solution containing Gabapentin and Nortriptyline Hcl. The method precision was carried out for the analyte six times using the proposed method. Repeatability was measured by multiple injections of homogenous sample of Gabapentin and Nortriptyline Hcl. Accuracy was carried out by percentage recovery studies at three different concentration levels. To the pre-analysed samples solution of Gabapentin and Nortriptyline Hcl , a known amount of standard drug powder of Gabapentin and Nortriptyline Hcl were added at 50, 100, 150% level. Specificity is a procedure to detect quantitatively the analyte in presence of component that may be expected to be present in the sample matrix, while selectivity is a procedure to detect qualitatively the analyte in presence of components that may be expected to be present in the sample matrix. Sensitivity of the proposed method was estimated in terms of limit of detection (LOD) and limit of quantification (LOQ) and was determined using the formulae; LOD =3.3 $\boldsymbol{\sigma}$ /S and LOQ = 10 $\boldsymbol{\sigma}$ /S, where, $\boldsymbol{\sigma}$ is the standard deviation 0f response and S is the slope of the calibration curve.

Robustness was evaluated by making deliberate variations such as variation of wavelength, flow rate and change in mobile phase composition. The robustness of the method was studied for Gabapentin and Nortriptyline Hcl. Ruggedness of the method was performed by two different analysts using same experimental and environmental conditions. It was performed by injecting 200µg/ml of GABAP and 5µg/ml solutions of NTH, respectively.The system suitability parameters such as resolution; number of theoretical plates and tailing factor were studied.

Stability of sample solution was established by the storage of sample solution at 25^{oc} for 12hr and 24hrs. Sample solution was reanalysed after 12 hrs and 24 hrs time intervals and assay was determined for Gabapentin and Nortriptyline Hcl and compared against fresh sample.

Forced Degradation Studies we studied different stress conditions like acid degradation, alkali degradation, heat degradation, hydrolysis, peroxide degradation, reduction degradation, thermal degradation and photo degradation. From all the degradation studies the maximum degradation for Gabapentin is 29.4 at humidity, NortriptylineHcl is 27.9 at alkali Linear correlation was obtained between peak area Vs concentration of Gabapentin and NortriptylineHcl were within the range. The linearity of the calibration curve was validated by the high value of correlation co-efficient of regression equation.

Analysis of Formulation

To determine the content of GABAP and NTH in injection formulation (GABAP 400mg, NTH 10mg). Weighed 10 tablets and crushed to powdered then 5 tablets equivalent of sample was taken into a 250 ml volumetric flask. Added 200 ml of diluent sonicated to dissolve and diluted to volume with diluent. Further diluted 5 ml to 100 ml with the diluent. Filtered through 0.45µ Nylon syringe filter. From the filtrate a 5mL solution was transferred into 50 mL volumetric flask and volume was made up to the mark with diluent to obtain a concentration of 400µg/mL of GABAP and 10µg/mL of NTH which was then subjected to proposed method and the amounts of GABAP and NTH were determined using calibration curves.

RESULTS

The proposed chromatographic system was found suitable for effective separation and quantization of GABAP (RT 3.457 min) and NTH (RT 4.728min) with good resolution, peak

shapes and minimal tailing. The overlay UV spectra and typical chromatogram were shown in Figures 1 and 2.

Both the drugs were found to give linear detector response in the concentration range under study with correlation coefficient of 0.999 for both GABAP and NTH. The GABAP and NTH have followed linearity in the concentration range of 50-500 µg/ml and 1-12.5 µg/ml respectively Figure 3.Percent recoveries for GABAP and NTH were 100.2-100.3% and 100.3-100.4 %RSD for tablet dosage form analysis, recovery studies and intra and inter-day precision studies was less than 2. LOD and LOQ were found to be 4.005µg/ml and 12.015 µg/ml for GABAP & 0.103 µg/ml and 0.309 µg/ml for NTH.

The method precision and inter-day precision were evaluated on the basis of % RSD value and found to be in the range 0.4224 -0.480 and 0.0883-0.2249 %. As the RSD values were < 2%, the developed method was found to be precise (Table 1). The accuracy of the method studied at three different concentration levels i.e. 50, 100, 150% showed acceptable recoveries in the range of 100.2-100.3%for GABAP and 100.3-100.4% for NTH (Table2).
The LOD for GABAP and NTH was found to be 4.005 and 0.103µg/ml respectively. Further the LOQ for GABAPand NTH was found to be 12.015 and 0.309µg/ml respectively Robustness of the method was studied by making deliberate changes in the chromatographic conditions like flow rate (± 0.2 ml/min) and mobile phase composition (± 5%). The validation parameters were summarized in (Table 3).

The results of robustness study of the developed method was validated by change in flow rate and change in mobile phase ratio and the % RSD of those variations are less than 2 (Table 4).

When the method was performed by two different analysts under the same experimental and environmental conditions it was found to be rugged and % RSD (<2%) indicating ruggedness of the method. The system suitability parameters such as number of theoretical plates and tailing factor were studied and shown in (Table 3).

Stability of sample solution was established by the storage of sample solution at 250^{OC} for 6hr, 12hr and sample was reanalysed after 24 hr and assay was determined for the compounds (GABAP and NTH) and compared against fresh sample. Sample solution did not show any appreciable change in assay value (% RSD<2) when stored at ambient temperature up to 24 hrs.

Six replicates of sample solutions containing 200µg/ml for GABAP and 5µg/ml for NTH were injected for quantitative analysis. The amounts of GABAP and NTH estimated were found to be 100.2 and 100.4% respectively. A good separation and resolution of both drugs indicates that there was no interference from the excipients commonly present in pharmaceutical combined dosage formulations. The results were shown in (Table 5).

Table 1: Precision of Developed Method.

S. No	Method precision				System precision			
	Gabapentin		NortriptylineHcl		Gabapentin		NortriptylineHcl	
	Rt (min)	Area	Rt (min)	Area	Rt (min)	Area	Rt (min)	Area
1	3.435	1674791	4.787	654951	3.564	1720815	4.782	629435
2	3.432	1672900	4.789	657348	3.553	1712733	4.781	634348
3	3.424	1672241	4.787	656711	3.553	1707928	4.789	629068
4	3.420	1671581	4.787	655694	3.533	1710647	4.780	625118
5	3.485	1674226	4.782	657289	3.526	1705437	4.779	630921
6	3.564	1671036	4.782	653607	3.524	1699406	4.785	628623
Mean	3.528	1672796	4.712	655933	3.521	1709494	4.725	629586
%RSD	0.125	0.0883	0.214	0.2249	0.081	0.4224	0.05	0.480

Table 2: Accuracy Data

Spike Level	Area	Amount of sample added (µg/ml)	Amount of API added (µg/ml)	Amount found (µg/ml)	%Recovery	%RSD
Gabapentin						
50%	899100	400	200	600	100.0	Mean 100.3
	895230	400	200	598	99.5	SD 0.24
	896862	400	200	604	100.2	% RSD 0.240
100%	1681242	400	400	799	99.75	Mean 100.5
	1685112	400	400	800	100.0	SD 0.08
	1688568	400	400	804	100.2	% RSD 0.084
150%	2703292	400	600	1000	100.0	Mean 100.2
	2697482	400	600	998	99.4	SD 0.12
	2704907	400	600	1000	100.0	% RSD 0.121
Nortriptyline Hcl						
50%	348389	10	5	15	100.0	Mean 100.4
	346869	10	5	14.9	99.9	SD 0.05
	346557	10	5	15.3	103	% RSD 0.051
100%	655186	10	10	20	100.0	Mean 100.2
	650353	10	10	19.8	98.0	SD 0.16
	655694	10	10	19.97	99.7	% RSD 0.164
150%	1061109	10	15	24.9	99.0	Mean 100.3
	1059668	10	15	25	100.0	SD 0.11 % RSD 0.111
	1057755	10	15	24.97	99.8	

Table 3: Validation and System Suitability Parameters.

Parameter	Gabapentin	Nortriptyline Hcl
Range (µg/ml)	50-500 µg/ml	1-12.5 µg/ml
Slope	5127	82405
Intercept	5127x+14084	82405x+28061
Correlation coefficient (R^2)	0.999	0.999
Retention time	3.457min	4.728 min
Precision (intra and inter day)% RSD	<2	<2
Accuracy	100.2-100.3	100.3-100.4
LOD(µg/ml)	4.005	0.103
LOQ(µg/ml)	12.015	0.309
Tailing factor	1.64	1.20
Theoretical plates	4037	6510
Resolution	-	5.90

Table 4: influence of flow rate, and mobile phase Composition on analytical parameters.

Parameter	Gabapentin			Nortriptyline Hcl		
	Rt (min)	Area	Tailing	Rt (min)	Area	Tailing
Flow rate(±0.2ml/min)						
1.0ml	4.182	2057310	1.81	5.744	805133	1.19
1.2ml	3.435	1674791	1.70	4.787	654951	1.20
1.4ml	2.904	1449536	1.22	4.090	569071	1.22
Mobile phase composition (±5%v/v)						
60:40	3.435	1674791	1.70	4.787	654951	1.20
55:45	2.566	1741745	0.92	4.085	679436	1.19
65:35	3.920	172663	1.85	5.115	676397	1.18

Table 5: Assay of Commercial Formulation.

Drug	Label claim(mg/tablet)	Calculated value (mg/tablet)	% of Assay
Gabapentin	400	400.3	100.2
Nortriptyline Hcl	10	10.5	100.4

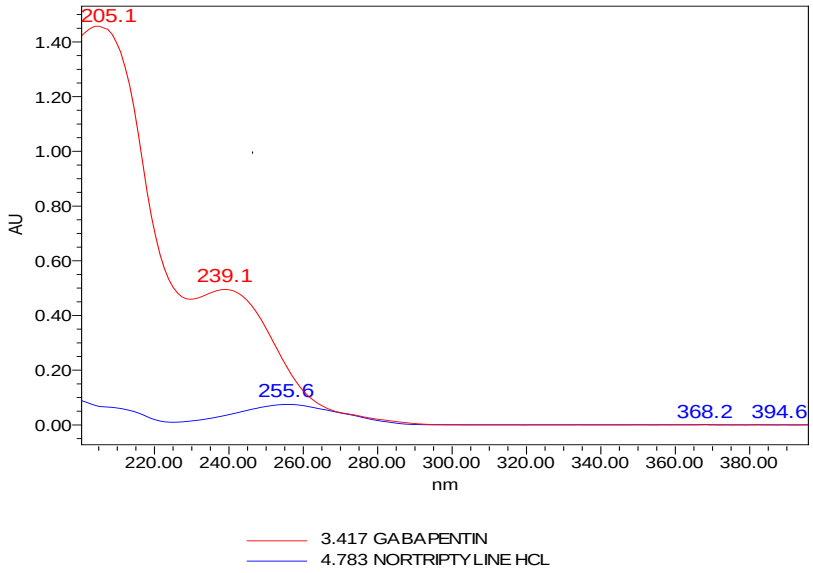


Figure 1: Overlay UV Spectra of Standard GABAP and NTH

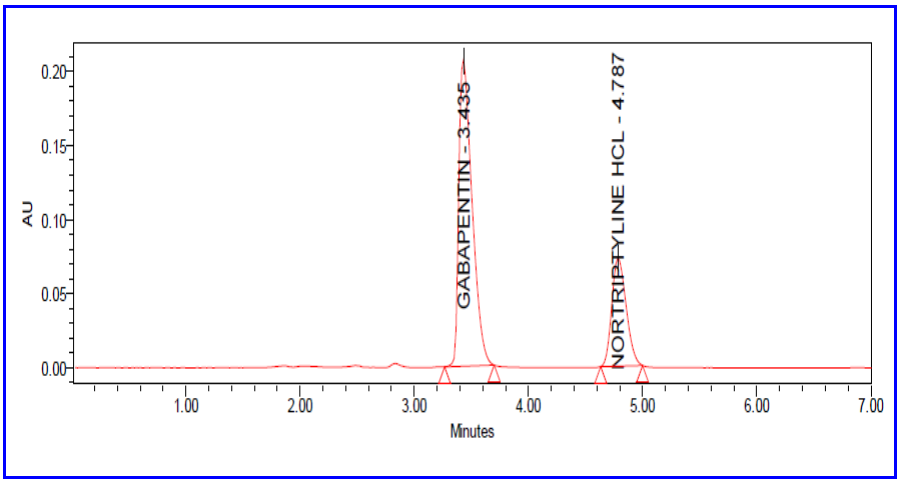
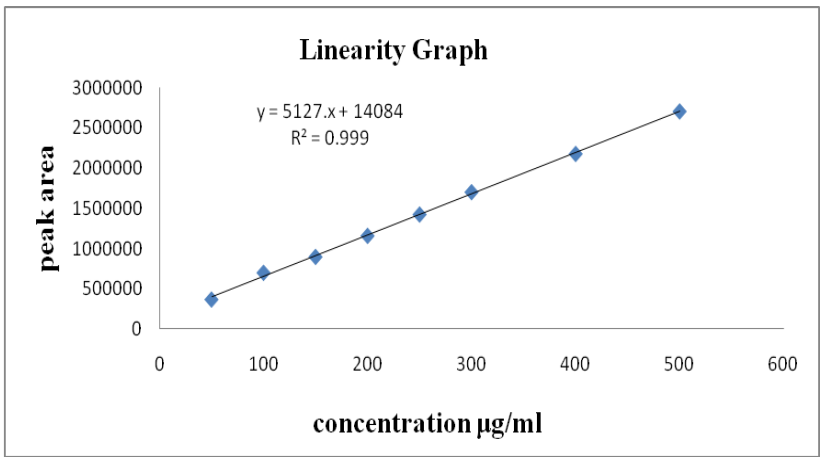


Figure2: Typical HPLC chromatogram ofGABAP and NTH



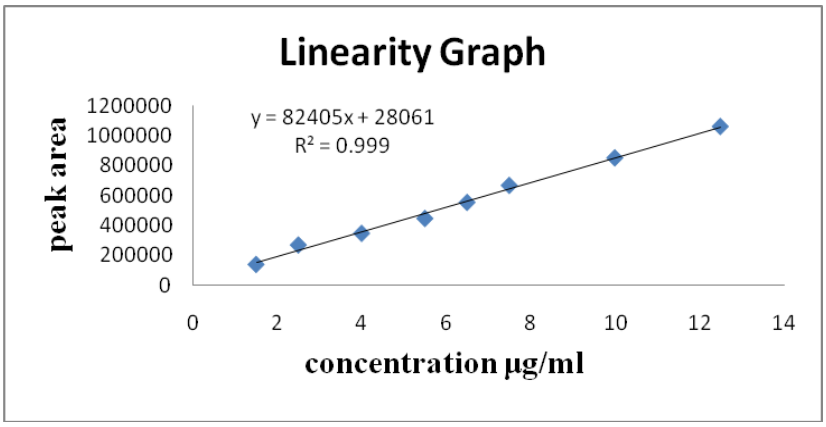


Figure 3: Calibration curves for GABAP and NTH

DISCUSSION

The developed RP-HPLC method was found suitable for simultaneous estimation of GABAP and NTH with good resolution, peak shapes and minimal tailing. The peak areas of the drug were reproducible as indicated by low coefficient of variance indicating the repeatability of the proposed method. High correlation coefficient of 0.999 showed the stable linear detector response in different concentration ranges of both the drugs.

The proposed method was validated as per ICH guidelines. The method exhibited good selectivity and sensitivity. Percent recoveries for GABAP and NTH were 100.2-100.3% and 100.3-100.4% respectively, indicating the accuracy of the proposed method. Low LOD and LOQ values indicate high sensitivity of the proposed method. The %RSD values of less than 2 for intra and inter day variation studies indicated that the proposed was precise. The developed method was studied for percentage recovery at three concentration levels and %RSD values of less than 2 were found which were in acceptable limits indicates the method was accurate. Low %RSD values of less than 2 in variation of flow rate and mobile phase ratio indicates the method was robust. When the method was performed by two different analysts under the same experimental and environmental conditions and %RSD was found to be less than 2 indicating the ruggedness of the proposed method.

The results from solution stability experiments confirmed that sample was stable up to 24 hr. during assay determination. The sample recoveries of GABAP and NTH from the commercial tablet dosage form were in good agreement with respective label claim indicating that there were no interferences from the commonly used tablet excipients and buffer used in analysis.

CONCLUSION

The low standard deviation and %RSD calculated for the proposed developed method and validation were in conformity with standards. Hence, it can be concluded that the developed RP-HPLC method is accurate, precise and selective and can be employed successfully for the simultaneous estimation of GABAP and NTH in tablet dosage form for routine quality control analysis.

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