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Research Article

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A Novel Spectrophotometric and RP-HPLC methods for Determination of Nortriptyline hydrochloride and Pregabalin in Tablets

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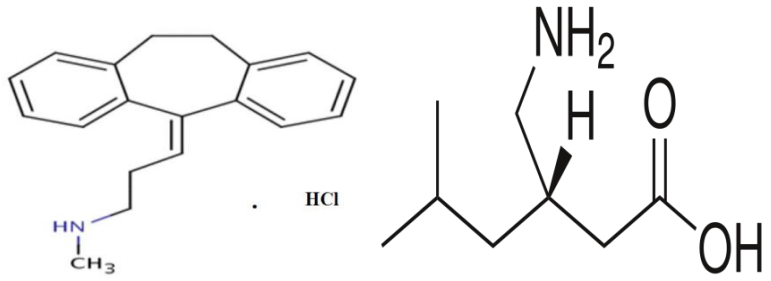
ABSTRACT

A Simple, rapid, specific, accurate, economical and precise UV spectrophotometric and RP-HPLC methods (in accordance with ICH guidelines) were developed and validated for determination of Nortriptyline hydrochloride and Pregabalin in tablet dosage form. The first method was based on Q - absorbance ratio, and absorbances of both drugs were determined at 239 nm (λmax of Nortriptyline Hydrochloride) and 235 nm (Iso-absorptive Point) when dissolved in methanol. It is found that Pregabalin does not have chromophoric group. To be UV-sensitive, it was compulsory to introduce chromophoric group in Pregabalin structure and make it UV-sensitive. This was achieved by converting the primary amine group of Pregabalin through reaction with benzoyl chloride to form benzoylated derivative of Pregabalin. Benzoylated Pregabalin was determined at 225 nm using UV-visible spectrophotometer. The second method was based on RP-HPLC. The chromatographic separation was performed on an Inertsil ODS C18 column (250 x 4.6mmx 5 µm) with a mobile phase of 0.56 %w/v Sodium hexane sulphonic acid dissolved in water acetonitrile (50:50 %v/v, pH 4.5 adjusted with Glacial Acetic Acid) at flow rate of 1.0 mL/min with DAD detection wavelength at 210 nm. Retention times of Nortriptyline Hydrochloride and Pregabalin were 7.3894 min and 4.0506 min, respectively. Beer-Lambert's law obeyed the concentration range of 2-12 µg/mL for Nortriptyline Hydrochloride and 10-60 µg/mL for Pregabalin. The results indicated that both spectrophotometric and RP-HPLC methods were linear, accurate, precise and robust with RSD values less than 0.2% and % recovery was within the standard limits (99 - 102%).

Key words: *Q-absorbance ratio method, RP-HPLC, Nortriptyline hydrochloride, Benzoylated derivative of Pregabalin, ICH guideline, Validation.*

INTRODUCTION

Nortriptyline Hydrochloride which is chemically known as 3-(10, 11-dihydro-5H-dibenzo [a, d] cyclohepten-5-ylidene)-N-methyl-1-propanamine hydrochloride belongs to a general class of tricyclic antidepressant drugs. Pregabalin is chemically (S)-3- (amino methyl)-5-methylhexanoic acid. It comes under the class of anticonvulsant [1-5].



Structure of Nortriptyline Hydrochloride **Structure of Pregabalin**

Figure 1. Nortriptyline Hydrochloride and Pregabalin Structure.

Combined treatment with Pregabalin and Nortriptyline hydrochloride is better than either agent alone for control of pain from diabetic polyneuropathy or postherpetic neuralgia which has been proven in clinical studies of Pregabalin in combination with an antidepressant drug Nortriptyline hydrochloride. In cases monotherapy does not result in adequate pain control, combined therapy should be considered. The combination of Nortriptyline Hydrochloride and Pregabalin is used effectively as antidepressant, anticonvulsant and to overcome neuropathic pain [6].

Through literature, several methods for NRT and PGB in single and in combined dosage form with other drugs using UV, HPLC and HPTLC determination have been reported. A stability indicating RP-HPLC method was reported for determination of NRT and PGB in combined dosage form [7, 8]. So, an attempt was made to develop simple, rapid, specific, accurate, precise, and economical Q - Absorbance Ratio method and simple new RP-HPLC method for determination of NRT and PGB in tablet formulations and both methods were validated as per ICH guidelines [9-13].

MATERIALS AND METHODS

Instrumentation

UV Spectrophotometry

Double beam UV-visible spectrophotometer (Shimadzu, model-1800, Japan) having two matched quartz cells with 1 cm light path was used for spectral measurements. UV probe 2.42 software was loaded on to UV-visible spectrophotometer.

Reverse phase-high pressure liquid chromatography

Instrumentation

HPLC system used was an Agilent High Pressure Liquid Chromatography 1260 equipped with Prominence DAD. Data were collected and processed using Ezchrome from Agilent. Software, Manual injector of 20-µl loop, Column - Inertsil ODS 3V C18 (250mm × 4.6mm), 5µ (particle size), Digital pH meter (Analab Scientific instruments Pvt. Ltd.) were also used.

Chemicals and Reagents

Nortriptyline hydrochloride and Pregabalin standard gift samples were provided by Zydus Cadila healthcare ltd., Ahmedabad, India. Methanol, acetonitrile and water (HPLC grade) were purchased from Merck Chemical Company. Sodium hexane sulphonic acid, glacial acetic acid (Analytical grade) and 0.22 µm pump Nylon filter were purchased from S.D. Fine Chemicals Ltd., Mumbai. The Whatman filter paper No. 41 was obtained from Modern Science Lab. All other reagents used were analytical grade. All the glassware used were borosilicate glass. Tablet formulations (PREGABID NT) were obtained from the local market.

Chromatographic conditions

Drugs were analyzed using an Inertsil ODS 3V C18 column (250mm × 4.6mm, 5µ) and Mobile phase was 0.56%w/v Sodium Hexane Sulphonic acid in water: Acetonitrile (50:50 %v/v, pH 4.5 adjusted with Glacial Acetic Acid). The Mobile phase was filtered through 0.22 µm Millipore membrane and degassed by ultra sonicator. A flow rate of 1.0 mL/min with an injection volume of 20 µl and detection wavelength of 210 nm was used.

Preparation of Solutions

Benzoylation of Pregabalin [7]

Stock solution of 1mg/mL Pregabalin was prepared in Methanol. Aliquot of 1 part of Pregabalin was allowed to react with 1 part of Benzoyl chloride. Few drops of Hydrochloride acid solution were added to precipitate out the final product. The product was filtered and dried. The optimum time of reaction completion was determined with TLC. The disappearance of the time spot of reactant from the product indicates formation of Benzoyl derivative. The benzoylated Pregabalin is easily quantified using UV Spectrophotometer as compared to parent moiety.

Preparation of standard solution (100 µg/mL)

10 mg of NRT and 10 mg of PGB were accurately weighed and transferred into 50 mL volumetric flask. About 25 mL methanol was added to dissolve it completely (sonicate if necessary) and the volume was made up with Methanol and filtered with 0.22 µm filter. The aliquots of the above stock solution were diluted further with Methanol to get concentrations of 2- 12 µg/mL for Nortriptyline hydrochloride and 10 – 60 µg/mL for Pregabalin.

Preparation of the sample solution

Twenty tablets of formulations (PREGABID NT containing 10 mg of Nortriptyline hydrochloride and 75 mg of Pregabalin) were accurately weighed to find out the mean weight and finely powdered. The tablet Powder equivalent to about 10 mg of Nortriptyline hydrochloride and 75 mg of Pregabalin was weighed and transferred into 100 mL volumetric flask and sonicated for 20 minutes. The solution was filtered through 0.22 µm Nylon filter paper to another 100 mL volumetric flask. Filtered solution was made up to the mark with methanol to get the final concentration of 100 µg/mL of NRT and 750 µg/mL of PGB. From this, 0.4 mL of solution was pipette out into 10 mL volumetric flask and made up to the mark with methanol to get the final concentration of 4 µg/mL of NRT and 30 µg/mL of PGB.

RESULT AND DISCUSSION

The main objective of this study was to develop a new HPLC – DAD and Q – absorbance ratio method for simultaneous analysis of NRT and PGB in tablet dosage form and validate it as per ICH guidelines.

In the Q – absorbance ratio method, the standard stock solutions (10 µg/mL) of NRT and PGB were scanned in the range of 200 – 400 nm against Methanol as a blank. Maximum absorbance was obtained at 239 nm and 225 nm for NRT and PGB, respectively. Iso-absorptive point was found to be at 235 nm. Calibration curves for NRT and PGB were plotted and molar absorptivity for both drugs were calculated at two wavelengths 239 nm (λmax of NRT) and 235 nm (Iso-absorptive Point) and concentration of NRT and PGB in the sample solutions were calculated using equation

$C_x = (Qm-Qy)*A1 / (Qx-Qy)*ax1$

$C_y = (Qm-Qx) *A1 / (Qy-Qx) *ay1$

Where C_x and C_y are concentrations of NRT and PGB, respectively. A1 and A2 are absorbances of sample solution at 235 nm (Iso absorptive Point) and 239 nm (λ_{max} of NRT). $Qx = ax_1/ax_2$ and $Qy = ay_2/ay_1$. ax_1 and ax_2 are molar absorptivity of NRT at 235 nm and 239 nm. ay_1 and ay_2 are molar absorptivities of Pregabalin at 235 nm and 239 nm [14].

In order to develop an isocratic RP- HPLC method, preliminary study for the analysis of the drugs in terms of parameters like detection wavelength, suitable mobile phase selection and optimum pH was carried out. The method was optimized to get good peak, resolution and other parameters. It was initially tried with different mobile phases like and finally the mobile was optimized based on separation efficiency achieved with 0.56%w/v Sodium Hexane Sulphonic acid in water: Acetonitrile (50:50 %v/v, pH 4.5 adjusted with Glacial Acetic Acid), pumped at the flow rate1.0 mL/min at 30°C. For determination of NRT and PGB in tablet, an Inertsil ODS 3V C18 column (250mm × 4.6mm, 5µ) was used. Retention time of NRT and PGB were 7.3894 min and 4.0506 min, respectively. The tailing factor, resolution and theoretical plates were found to be in compliance with the ICH guidelines [15].

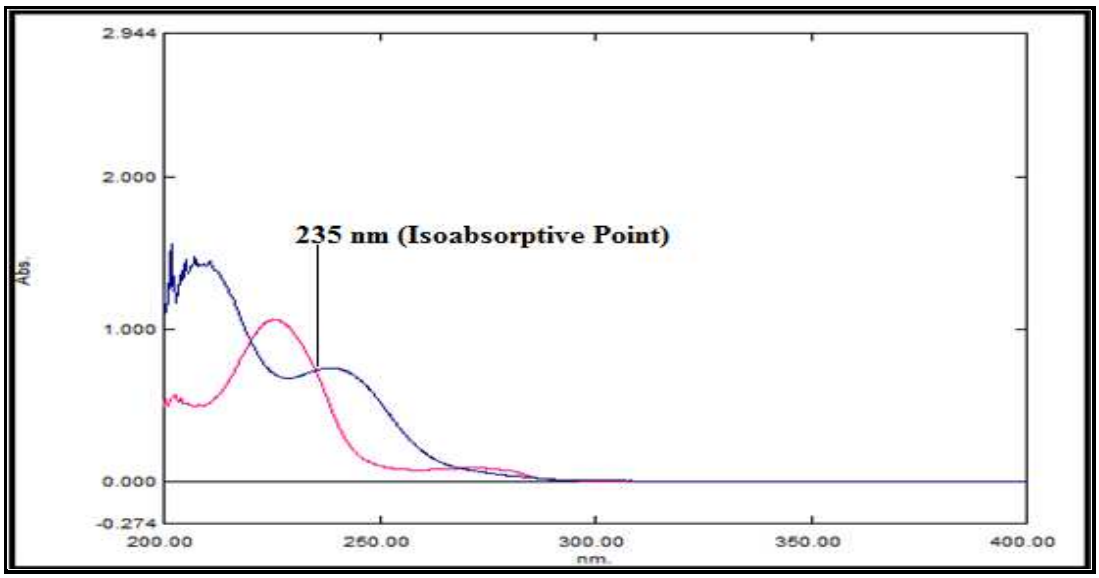


Figure 2. Overlay spectras of standard solution (10 µg/mL) of NRT and PGB

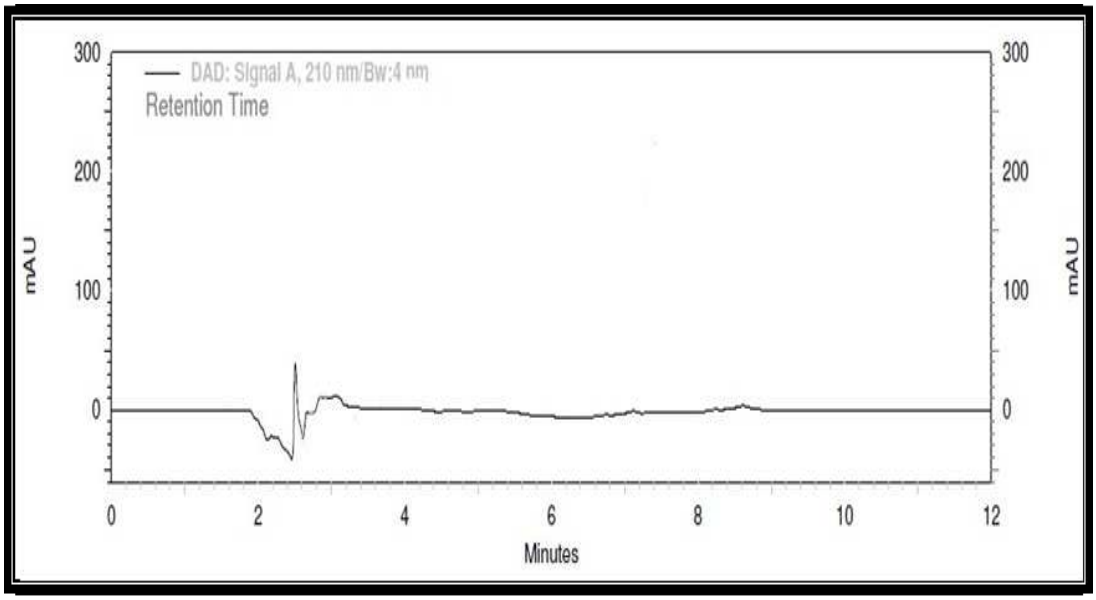


Figure 3. Chromatogram of mobile phase

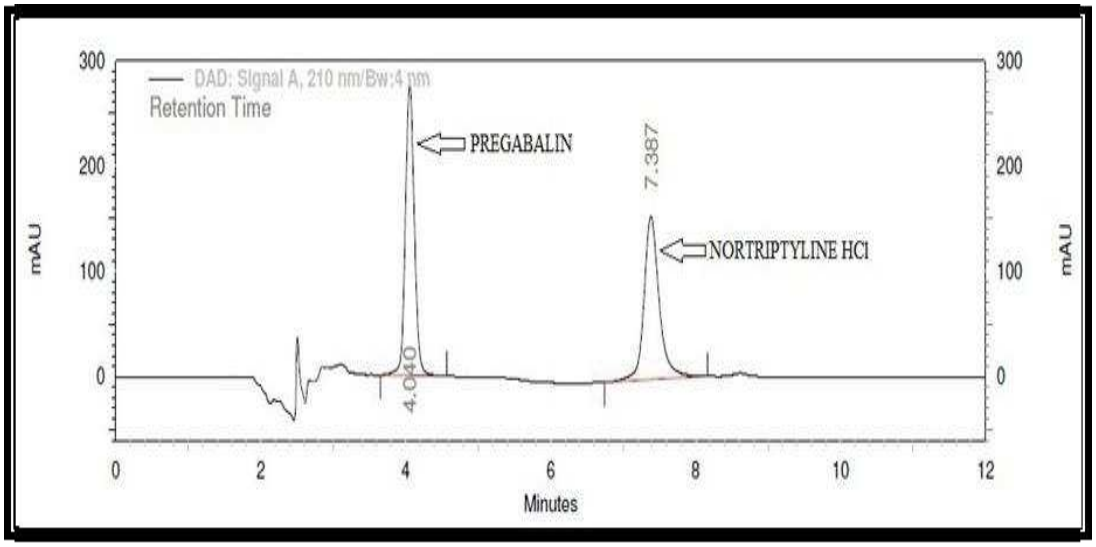


Figure 4. Chromatogram of standard solution of NRT and PGB

METHOD VALIDATION [15]

Linearity

The linearity was evaluated by analyzing different concentrations of the standard solutions of NRT and PGB and it was found to be linear in the range of 2- 12 µg/mL for NRT and 10-60 µg/mL for PGB. The visual characteristics such as linearity range, standard deviation on slope and intercept, correlation coefficient and regression linear equation were calculated, and are summarized in Table 1.

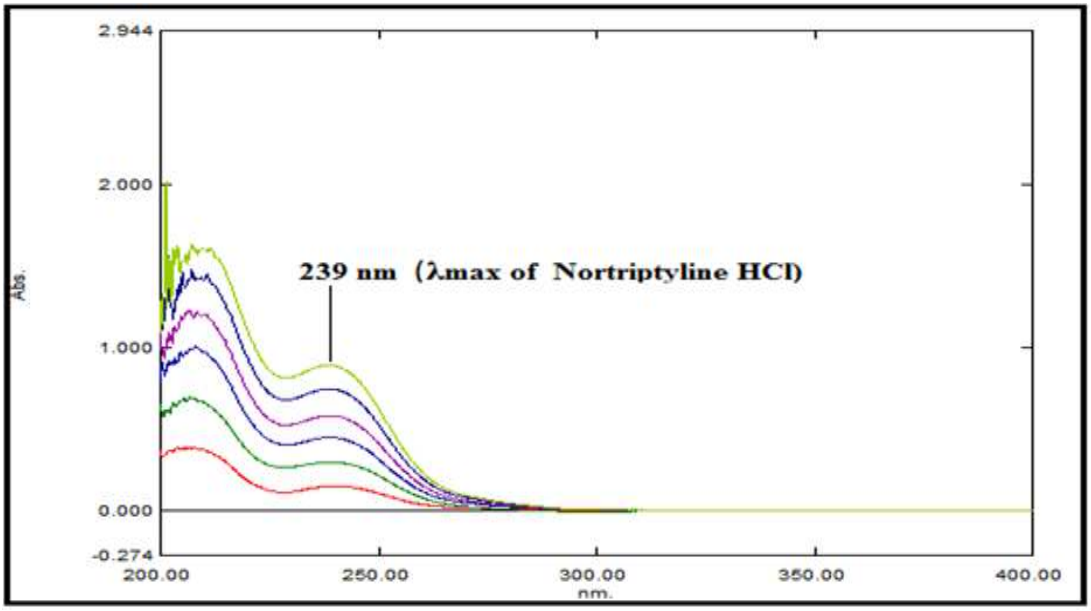


Figure 5. Absorption spectras of six different concentrations (2 – 12 µg/mL) of NRT

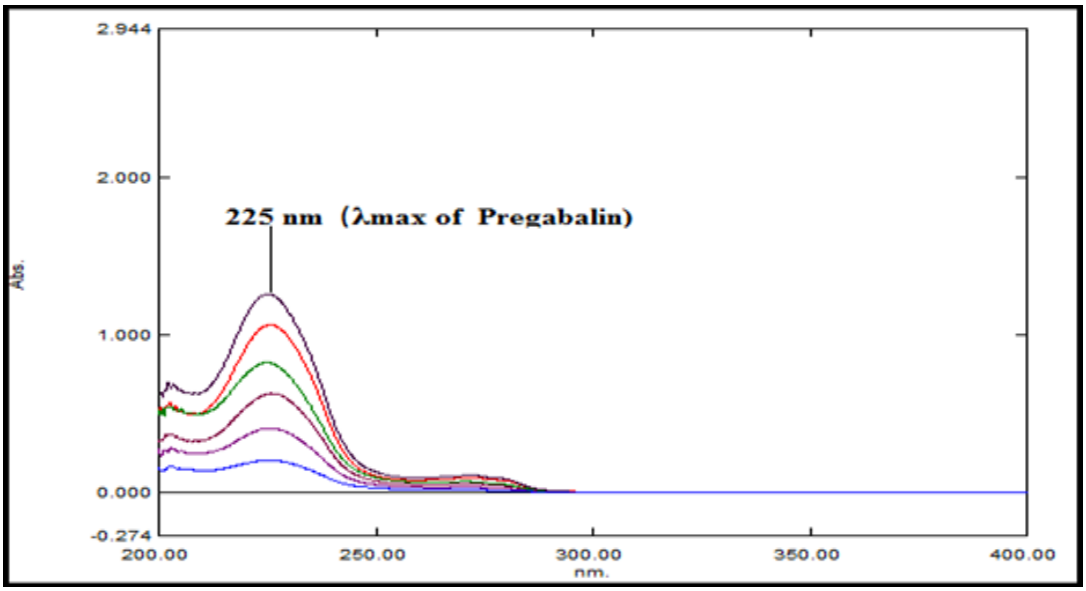


Figure 6. Absorption spectras of six different concentrations (10 – 60 µg/mL) of PGB

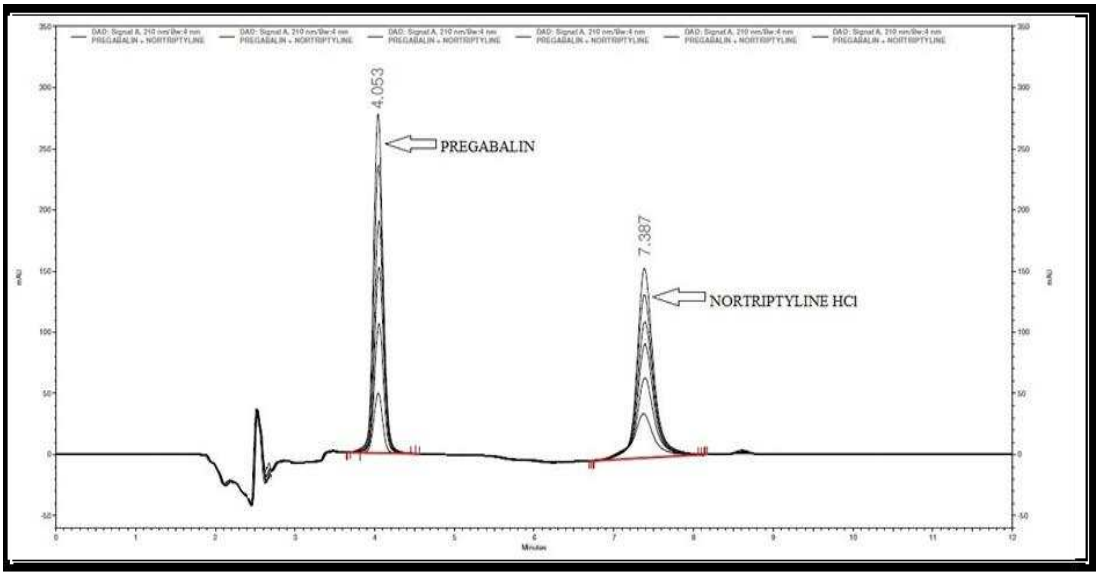


Figure 7. Overlay chromatograms of serial dilutions of NRT and PGB by RP-HPLC

Accuracy (%Recovery)

The accuracy of proposed method was determined by % recovery. Standard addition was used for accuracy determinations at three levels (80%, 100% and 120%) of concentrations; involving analysis of formulation of the samples containing 4 µg/mL of Nortriptyline hydrochloride and 20 µg/mL of Pregabalin to which certain amounts of authentic drugs were added. The recovering range for NRT and PGB were found to be 99% to 102% as shown in Table 2.

Precision

Method precision (Repeatability)

Repeatability was studied by calculating the RSD for six determinations of the concentration of about 6 µg/mL and 30 µg/mL for NRT and PGB, respectively, performed on the same day and under same experimental conditions. The corresponding results are shown in Table 1 and %RSD was found to be less than 1.0%.

Intermediate precision(Reproducibility)

Intraday and inter day variations were determined by six solutions (2-12 µg/mL) of NRT and (10-60 µg/mL) of PGB within the same day and three different days over a period of week. The corresponding results are recorded in Table 1 and %RSD was found to be less than 2.0%.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD and LOQ for both drugs were calculated theoretically using following equation as per ICH guidelines. These data show that the method is sensitive (Table 1).

LOD = 3.3 × σ/S and LOQ = 10 × σ/S

Where σ is the standard deviation of the response and S is the slope of the calibration curve.

Specificity

Specificity was performed to exclude the possibilities of interference with excipients in the region of elution of Nortriptyline hydrochloride and Pregabalin. The specificity of the method was tested under normal conditions and the result of the tests proved that the components other than the drug did not produce a detectable signal at retention place of NRT and PGB.

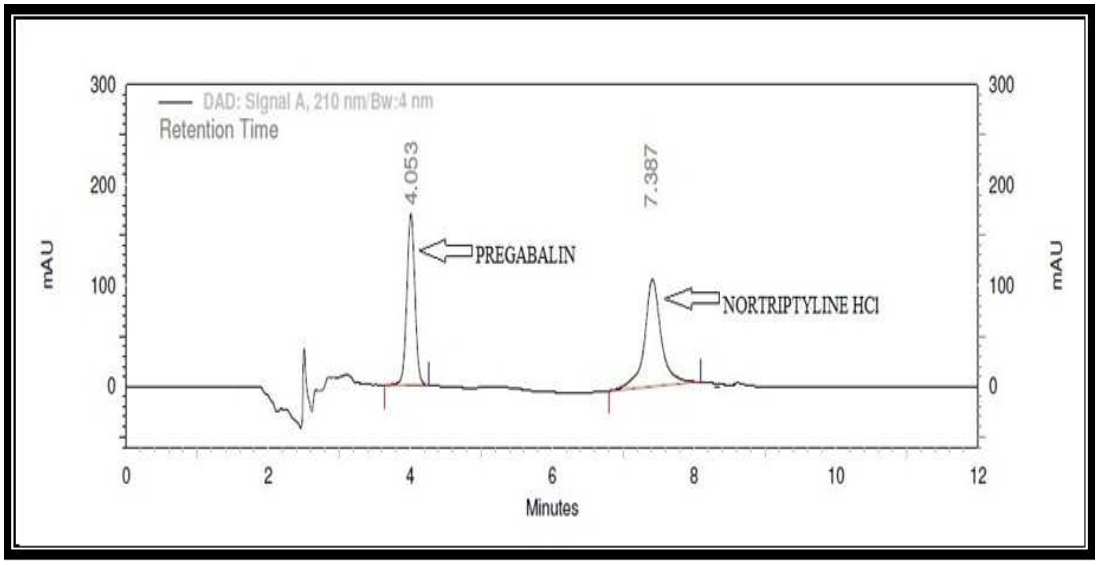


Figure 8. Chromatogram of sample solution of NRT and PGB by RP-HPLC

Robustness

The robustness of the method was verified by altering the chromatographic conditions like flow rate, temperature, pH, mobile phase and wavelength. The results are shown in Table 4.

System suitability

The system suitability test is used to verify that the resolution reproducibility of system is adequate for the analysis. Retention time, number of theoretical plates, tailing factor and resolution were calculated for NRT and PGB. The results are shown in Table 3.

Table 1. Summary of method validation parameters for NRT and PGB

Parameters		Q - Absorbance Ratio method				HPLC	
		NRT		PGB		NRT	PGB
		235nm	239 nm	235 nm	239nm		
Calibration range (µg/mL)		2 - 12	2 - 12	10 - 60	10 – 60	2-12	10-60
Molar Absorptivity ($1mole^{-1}cm^{-1}$)		717.38	297.91	144.62	92.60	-	-
Slope		0.0732	0.0743	0.0147	0.0093	323,209.35	83,416.98
Intercept		0.0075	0.0008	0.0060	0.0006	932,740.00	20,831.04
Correlation coefficient		0.9995	0.9998	0.9992	0.9994	0.9994	0.9993
Precision (%RSD)	Intra-day	0.09-0.41	0.09-0.38	0.12-0.82	0.10-0.62	0.11-0.56	0.19-0.49
	Inter-day	0.17-1.08	0.31-1.40	0.06 –0.81	0.10-0.61	0.16-0.78	0.29-0.56
	Repeatability	0.094	0.114	0.167	0.221	0.30	0.14
LOD (µg/mL)		0.026	0.023	0.304	0.236	0.03682	0.20303
LOQ (µg/mL)		0.078	0.069	0.923	0.715	0.11157	0.61523

*NRT: Nortriptyline hydrochloride and PGB: Pregabalin

Table 2. Accuracy studies of NRT and PGB (% Recovery)

Drug	Spiked level (%)	Amount taken (µg/mL)	Amount added (µg/mL)	Amount found (µg/mL) ± S.D (n=3)		% Recovery ± S.D (n=3)	
				Q-abs	HPLC	Q-abs	HPLC
NRT	80	4	32	7.32 ± 0.05	7.20±0.01	101.6±0.71	100.03±0.10
	100	4	4	8.08 ± 0.05	8.09± 0.01	100.8±0.51	101.19±0.16
	120	4	4.8	8.92 ± 0.05	8.80± 0.02	101.4±0.60	100.08±0.23

PGB	80	20	16	36.19±0.10	36.00±0.25	100.5±0.28	100.02±0.69
	100	20	20	40.04±0.15	40.35±0.07	100.1±0.38	100.89± 0.19
	120	20	24	44.18±0.16	44.49±0.15	100.4±0.37	101.12±0.34

Table 3. System Suitability parameters of RP-HPLC method

Parameter	PGB	NRT
Retention time(Minutes)	4.0506	7.3894
Theoretical plates (T_P)	23276.8	28659.4
Tailing factor (T_f)	1.022	1.008
Resolution(Rs)	6.572	11.746

Table 4. Robustness studies of NRT and PGB

Parameter	Modification	NRT % Recovery± % RSD (n=3)	PGB % Recovery ± % RSD (n=3)
Flow rate (1 mL/min)	0.9mL/min	100.03 ± 0.88	100.85 ± 0.72
	1.1mL/min	101.36 ± 0.60	100.78 ± 0.36
Mobile phase Water : ACN (50:50 v/v)	48:52 v/v	100.74 ± 0.45	101.07 ± 0.68
	52:48 v/v	101.08 ± 0.43	100.94 ± 0.60
Wavelength (210nm)	209 nm	99.99 ± 0.54	101.22 ± 0.36
	211 nm	100.68 ± 0.73	100.57 ± 0.37
pH (4.5)	4.3	101.72 ± 0.26	101.34 ± 0.36
	4.7	100.18 ± 0.80	101.04 ± 0.42
Temperature (30°C)	28°C	100.66 ± 0.42	99.87 ± 0.31
	32°C	100.77 ± 0.59	100.37 ± 0.47

Table 5. Analysis of marketed formulations by proposed methods

Parameter	Q-abs		RP-HPLC	
	NRT	PGB	NRT	PGB
Label Claim(mg/tablet)	10	75	10	75
Assay(Content in mg)	10.16	75.22	10.04	75.37
% Assay(Mean* ± S.D, n=6)	101.6 ±0.32	100.3±1.14	100.4 ± 0.41	100.5 ±0.28

*NRT = Nortriptyline hydrochloride
*PGB = Pregabalin
*Q - abs = Q - Absorbance Ratio method

CONCLUSION

The Q-Absorbance Ratio and RP-HPLC method for simultaneous determination of Nortriptyline hydrochloride and Pregabalin in their tablet dosage form were developed and validated as per ICH guidelines. Linearity was observed in the range of (2-12 µg/mL) for NRT and (10-60 µg/mL) for PGB with correlation coefficient ($r^2 = 0.999$). The % recoveries of NRT and PGB were in the range of 99-102% which was within the acceptance criteria. The %RSD was not more than 2% which proved the precision for the developed method. By studying all the validation parameters (Linearity, Precision, Accuracy, LOD, LOQ, Robustness and System Suitability parameters), we have concluded that the methods were simple, precise, accurate, rapid and robust for the determination of Nortriptyline hydrochloride and Pregabalin in their tablet dosage form. The assay results showed that the method can be

successfully applied for routine analysis of NRT and PGB in tablet dosage form.

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