Chemical Methodologies 3(2019) 494-508

Chemical Methodologies

Journal homepage: http://chemmethod.com

Original Research article

Validated Stability Indicating RP-HPLC DAD Method for Simultaneous Determination of Amitriptyline Hydrochloride and Pregabalin in Presence of Stress degradation products in tablet dosage form

Amit J. Vyas, Grishma P. Nathwani*, Ajay I. Patel, Nilesh K. Patel, Ashok B. Patel

B. K. Mody Government Pharmacy College, Rajkot-360002, Gujarat, India

ARTICLE INFORMATION

Received: 1 November 2018
Received in revised: 13 December 2018
Accepted: 23 February 2019
Available online: 11 March 2019

DOI: 10.22034/chemm.2019.154554.1107

KEYWORDS

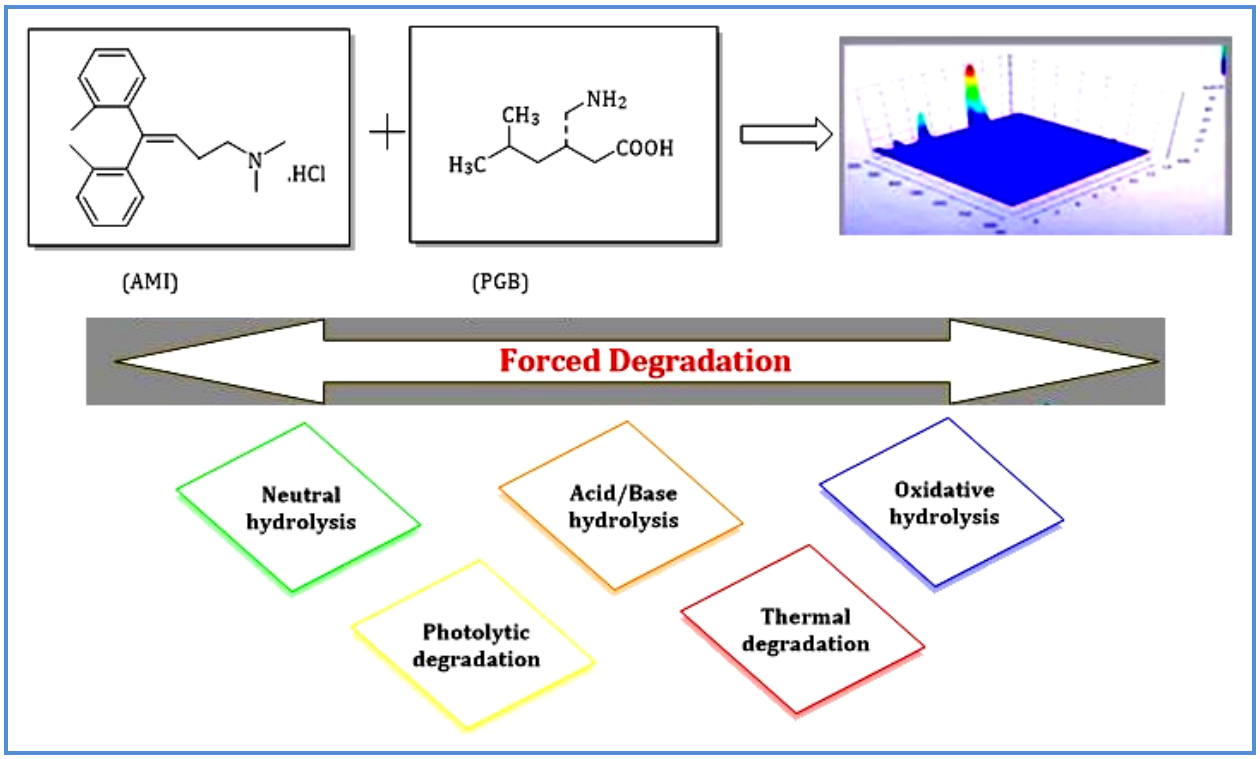
Amitriptyline hydrochloride
Pregabalin
Stability indicating method
RP-HPLC
Method validation

ABSTRACT

Background: High Performance liquid chromatography (HPLC) is an integral analytical tool in assessing drug product stability. HPLC methods should be able to separate, detect, and quantify the various drug-related degradants that can form on storage or manufacturing, plus detect any drug-related impurities that may be introduced during synthesis.
Objectives: A simple, economic, selective, precise, accurate and stability indicating RP-HPLC (Reversed phase–HPLC) method was developed and validated for analysis of Amitriptyline Hydrochloride (AMI) and Pregabalin (PGB) in the formulation.
Method: Reversed-phase chromatography was performed on a C_{18} column with buffer (potassium dihydrogen phosphate) pH 4.0 and acetonitrile, (40:60 %v/v), as mobile phase at a flow rate of 1 mL/min.
Result: The detection was performed at 230 nm (nanometer) and sharp peaks were obtained for PGB and AMI at retention time of 4.4 and 9.6 min, respectively. The detection limits were found to be 3.00 µg/mL and 0.59 µg/mL and quantification limits were found to be 9.11 µg/mL and 1.79µg/ml for Pregabalin and amitriptyline hydrochloride, respectively. The method was validated for accuracy, precision, reproducibility, specificity, robustness and detection and quantification limits, in accordance with ICH (international council of hormonization) guideline.
Conclusion: Stress study was performed on Pregabalin and amitriptyline hydrochloride and it was found that these degraded sufficiently in all applied chemical and physical conditions. Thus, the developed RP-HPLC method was found to be suitable for the determination of both the drugs as well as stability samples of tablets containing various excipients.

**Corresponding author: E-mail: grishmanathwani172gamil.com, B. K. Mody Government Pharmacy College, Rajkot-360002, Gujarat, India, Tel: +9428758511*

Graphical Abstract



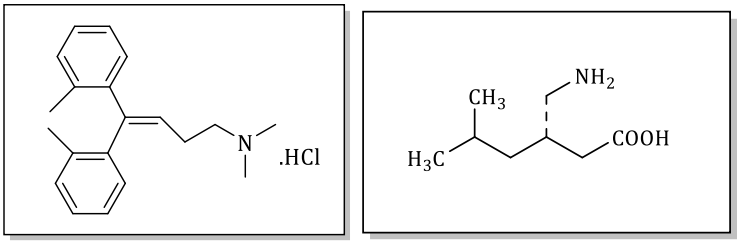
Introduction

Chemical stability of pharmaceutical molecules is a matter of great concern as it affects the safety and efficacy of the drug product. The FDA and ICH guidelines state the requirement of stability testing data to understand how the quality of a drug substance and drug product changes with time under the influence of various environmental factors [1]. Knowledge of the stability of molecule which helps in selecting proper formulation and package as well as providing proper storage conditions and shelf life is essential for regulatory documentation. Forced degradation is a process that involves degradation of drug products and drug substances at conditions more severe than accelerated conditions and thus generates degradation products that can be studied to determine the stability of the molecule [2]. The ICH guideline states that stress testing is intended to identify the likely degradation products which further helps in determination of the intrinsic stability of the molecule and establishing degradation pathways, and to validate the used stability indicating procedures [3].

Amitriptyline hydrochloride (AMI), chemically 3-(10,11-dihydro-5*H*-dibenzo[a,d]cyclohept-5-ylidene)propyl dimethyl amine hydrochloride, is a tricyclic antidepressant. It inhibits the reuptake

of nor epinephrine and serotonin by the presyneptic neuronal [4]. The structure of amitriptyline hydrochloride is shown in (Figure 1) [5]**.**

Pregabalin (PGB), chemically (S)-4-amino-3-(2-methyl propyl) butyric acid, which is analogue of gabapentin, is more potent but very similar. It is used as anti epileptic by binding with high affinity to the alpha-2-deltasite (subunit of calcium channels) [4]. The structure of pregabalin is shown in (Figure 1) [5]**.**



(AMI) **(PGB)**

Figure 1. Structure of AMI & PGB

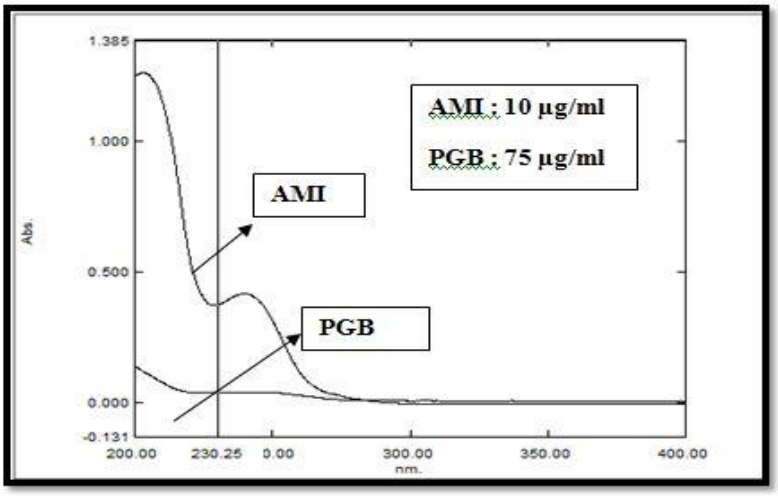


Figure 2. UV Overlay Spectra of AMI & PGB

Amitriptyline HCl (AMI) and pregabalin (PGB) are available as tablet for treatment against neuropathic pain. The literature survey of AMI and PGB reveals various analytical methods which are available for the determination of AMI and PGB both individually and in other combination methods. Moreover, various analytical methods like RP-HPLC [6-13], HPTLC [14], spectro photometric methods [15-17], stability indicating HPLC method for single drug [18-20] and LC-MS methods [21-24] are available. However, no stability indicating RP-HPLC method is reported for combination in the literature. Thus, in present study it was decided to develop stability indicating

RP-HPLC method for simultaneous estimation of AMI and PGB in tablet dosage form. The method was validated in compliance with ICH guideline (Q2 R1) [25].

The main objective to perform this study was regulatory requirement. Current FDA and ICH guideline recommends inclusion of the results, including chromatograms of stressed samples, demonstration of the stability-indicating nature of the analytical procedures, the associated procedures, and the degradation pathways of the API in solid state, solution, and drug product [26].

A new rapid, selective, precise, accurate stability indicating RP-HPLC method for Amitriptyline Hydrochloride and pregabalin in presence of the generated degradants was developed. This concurrent determination of both drugs in the presence of their degradants offers wide scope of applications and therefore is useful in practice.

Material and Methods

Amitriptyline hydrochloride was kindly gifted by medicamen biotech limited and pregabalin was kindly gifted by Reine Lifescience. Combined dose of amitriptyline hydrochloride and pregabalin tablets (maxgalip at, 10 mg amitriptyline hydrochloride and 75 mg pregabalin manufactured by Sun Pharmaceuticals) were taken. De-ionized water (HPLC grade) was used, supplied by Loba chemie (Mumbai, India) throughout the experiments. Acetonitrile (HPLC grade) was procured from Finar (Ahmedabad, India). Potassium dihydrogen phosphate, ortho phosphoric acid (all of analytical grade) was procured from Molychem (Mumbai, India).

Preparation of standard solution

Accurately weighed 5 mg of Amitriptyline Hydrochloride and 10 mg of pregabalin were transferred to a 50 mL amber colour volumetric flask, sufficient amount of acetonitrile was added and sonicated to dissolve it and volume was made up to 100 mL (Standard contains: 100 µg/mL of AMI and 200 µg /mL of PGB). Aliquots of 0.4, 0.6, 0.8, 1.0, 1.2, and 1.4 mL were taken to be further diluted with acetonitrile up to 10 mL to get a series of concentration of 4-14 µg/mL for AMI and 30-105 µg/mL for PGB, respectively.

Mobile phase selection

In order to select a suitable mobile phase for the analysis of AMI and PGB, various combinatorial ratios of various solvents were tried on the basis of trial and error method [27]. Considering the system suitability parameters viz. Retention time, tailing factor, the number of theoretical plates and HETP, and the mobile phase found acetonitrile to be most suitable for analysis: potassium dihydrogen phosphate buffer pH 4.0 in ratio of 60:40. The mobile phase was filtered through 0.45 µ

filter paper to remove particulate matter if any and then degassed by sonication. Flow rate employed for analysis was 1.0 mL/min and the concentrations were detected at 230 nm.

System suitability Parameters

The suitability of the chromatographic system was tested before each stage of validation. Six replicates injections of standard preparation were injected and retention time, Tailing factor, the number of theoretical plates, and relative standard deviation of peak area were determined.

Stress (forced decomposition) studies

Hydrolytic decomposition: Acidic and alkaline hydrolysis of AMI and PGB were carried out in 0.1M HCl and 0.1M NaOH at a concentration of 6 µg/mL of AMI and 45 µg/mL of PGB, respectively. Acidic condition was applied for 4 hrs and basic condition was applied for 2 hrs. The stressed samples were neutralized and diluted with the mobile phase and filtered through 0.45 µ filter.

Neutral decomposition: Neutral hydrolysis of AMI and PGB was carried out in distilled water at a concentration of 6 µg/mL of AMI and 45 µg/mL of PGB, respectively for 24 hrs.

Oxidative decomposition: Solutions for oxidative stress studies were prepared using 3% H_2O_2 at a concentration of 6 µg/mL of AMI and 45 µg/mL of PGB, respectively for 2 hrs. The sample solution was diluted according to the mobile phase.

Photolytic decomposition: Photo degradation studies were carried out by exposing the solid powdered drug to UV light in the UV chamber at the wavelength (254 nm) for 24 hrs.

Thermal decomposition: thermal studies were also conducted on solid drug, which was heated at 105 °C for 24 hrs in hot air oven. Peak purity was performed for all condition using PDA detector.

Stress (forced decomposition) studies on formulation

A quantity of the powder equivalent to 45 mg of PGB and 6 mg of AMI was transferred into 50 mL amber coloured volumetric flask and add 40 ml of water/acid/base/H_2O_2 solutions. The physic-chemical conditions were applied to the formulation. So, concentration of AMI was 150 µg/mL and PGB was 1125 µg/mL. Then, we sonicated this solution for 10 min and appropriately diluted it to have the final concentration 6 µg/mL AMI and 45 µg/mL PGB with acetonitrile.

Method Validation

Linearity

Aliquots of 0.4, 0.6, 0.8, 1.0, 1.2, and 1.4 mL were taken to be further diluted with acetonitrile up to 10 mL to get a series of concentration of 4-14 µg/mL for AMI and 30-105 µg/mL for PGB, respectively.

Repeatability

Repeatability expresses the precision under the same operating conditions over a short interval of time. Repeatability was performed under 6 replicates at concentration of 6 µg/mL of AMI and 45 µg/mL of PGB, respectively.

Precision

Precision was considered at two levels, *i.e.* repeatability and inter-mediate precision, in accordance to ICH recommendations. Repeatability of the sample injection was determined as intra-day variation whereas inter-mediate precision was determined by measuring inter-day variation for triplicate determination of AMI and PGB at three different concentrations (80, 100, 120 %) of AMI 4.6, 6, and 7.2 µg/mL and PGB 36, 45, and 54 µg/mL respectively. Results from determination of repeatability and intermediate precision, expressed as RSD (%).

LOD and LOQ

The limit of detection (LOD) and limit of quantification (LOQ) were calculated experimentally. Calibration curve was repeated for five times and standard deviation (SD) of the intercepts was calculated. The LOD and LOQ of the drug were derived by calculating the signal-to-noise (*i.e.* 3.3 for LOD and 10 for LOQ) ratio using following equations designated by International Council for Harmonization (ICH) guideline:

$LOD = 3.3 \times \sigma/S$ and $LOQ = 10 \times \sigma/S$

Where, σ = standard deviation of the response

S = Slope of the calibration curve.

Robustness

The robustness of method was established by introducing small changes in various parameters like flow rate, pH, and mobile phase composition. The changes made in flow rate, pH and mobile phase

composition were ± 0.1 mL/min (0.9, 1.0, 1.1 mL/min), ± 0.1 unit (3.9, 4.0, 4.1 unit), ± 2 mL (58:42, 60:40, 62:38 mL) respectively. The robustness of the method was evaluated by calculating % RSD.

Accuracy

Accuracy of the analytical method was determined by the involved systemic error. It is the closeness of test results obtained by that method to the true value. The accuracy of the method was carried out at three levels 80, 100 and 120% (10.8, 12, and 13.2 µg/mL for AMI and 81, 90, and 99 mcg/mL for PGB) of the working concentration of sample. Calculated amounts of standard solution of AMI and PGB were spiked with added sample solution to prepare level 80, 100 and 120% of the working concentration.

Assay of Marketed Formulation (n=6)

Tablet was accurately weighed and finely powdered. A quantity of the powder equivalent to 169.8 mg was transferred into 100 mL amber coloured volumetric flask. Then, we added 60 mL of diluent and sonicate for 15 min, made up volume up to the mark with diluents, filtered the solution and discarded the first 5 mL solution. Afterwards, we took 0.4 mL from the filtered solution in 10 mL volumetric flask and made up the volume up to the mark with diluent. Final concentration of AMI was 6 µg/mL and PGB was 45 µg/mL respectively.

Results and Discussion

The present stability-indicating method for the determination of AMI and PGB in tablet dosage form is specific because the drugs peak was well separated even in the presence of degradation products. Overall, the data demonstrated that the excipients and the degradation products did not interfere with the drugs peak, indicating the best selectivity of the method. Quantification was achieved with UV detection at 230 nm based on peak area shown in Figure 3. The main objective of the chromatographic method development was to separate the degradation products which were obtained from the stress studies from the drugs peak.

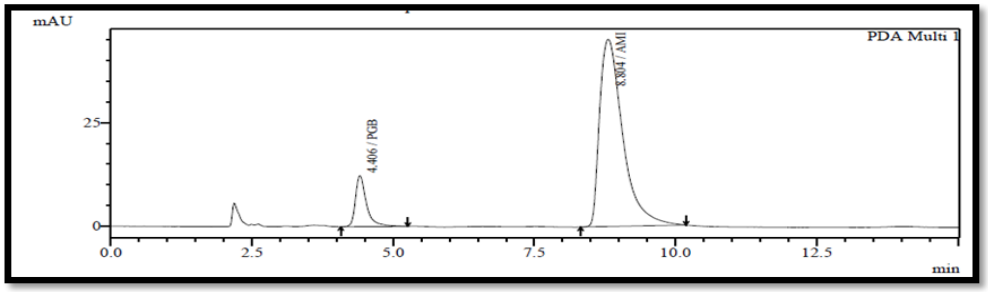


Figure 3. Chromatogram of optimized condition

HPLC method development and optimization

Optimizations of chromatographic conditions were performed to obtain the good peak, shape and peak parameter (tailing factor, theoretical plates). For the selection of mobile phase, initially, the different concentration of ACN: water and ACN: Phosphate buffer with different pH has been tried, but it gave poor peak shape and also poor system suitability parameters. Finally, ACN: potassium dihydrogen phosphate buffer (60: 40) mixture tried at pH 4.0 at a flow rate of 1 mL/min was found to be satisfactory with good system suitability parameter. Using these experimental conditions, all peaks were well resolved with good peak shape. Therefore, this mobile phase provided the best chromatographic response and was used for further studies.

The average retention time for PGB and AMI were found to be 4.4 and 8.6, respectively. The tailing factor for both the drugs were observed as less than 2 and theoretical plates for PGB and AMI were found to be more than 2000. So all system suitability parameters were complied as per USP. Chromatogram of optimized condition is shown in Figure 4**.** and results of system suitability is summarized in Table 1.

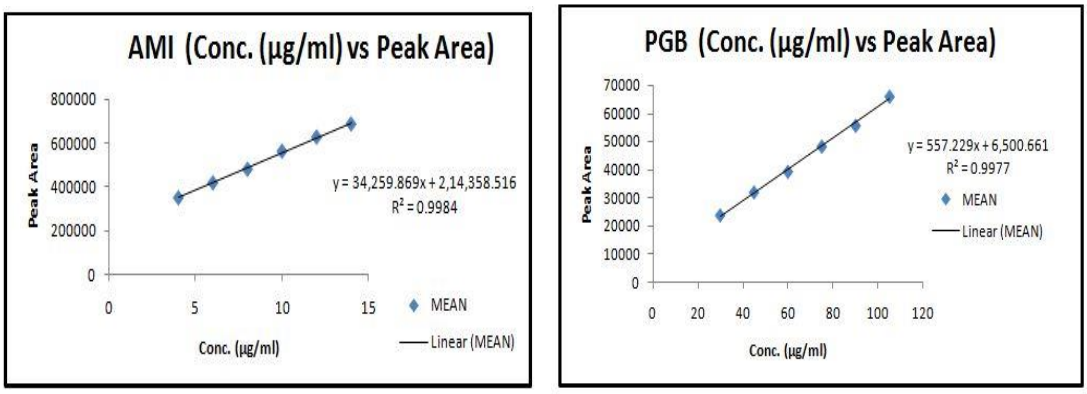


Figure 4. Calibration curve of AMI & PGB

Table 1. System suitability parameter

System suitability parameter	AMI	PGB
Retention times (Rt)	9.6 ± 0.2	4.4 ± 0.01
Theoretical plates (N)	2914 ± 40.00	3637 ± 77.26
Resolution (R_S)	10.44 ± 0.02	0.000
Tailing Factor	1.43 ± 0.01	1.13 ± 0.01

The calibration curves for PGB and AMI were linear over the concentration range of 30-105 µg/mL (for PGB) Figure 5**.** and 4-14 µg/mL (For AMI) Figure 6. The correlation coefficient values were found to be 0.9984 and 0.9977 for PGB and AMI, respectively. The results of correlation coefficients

are summarized in Table 2. The obtained areas were directly proportional to the concentration of analyte in the sample. The method can, therefore, be termed as linear in the range considered.

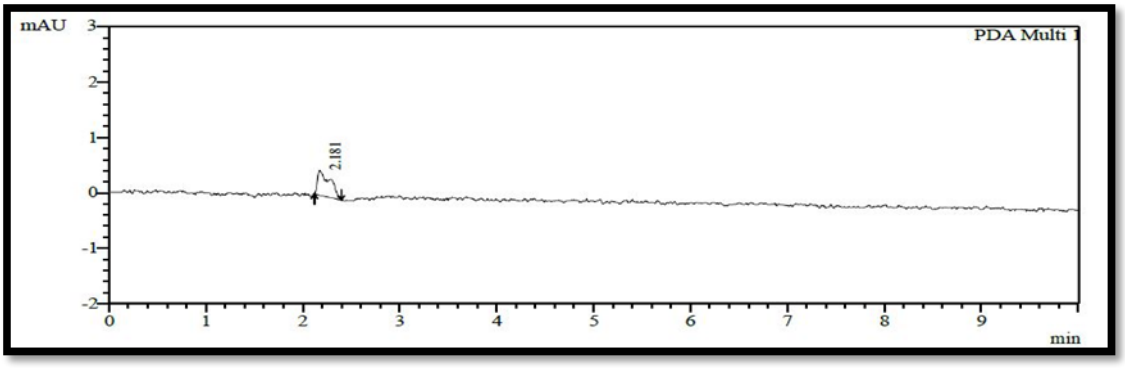


Figure 5. Chromatogram of blank (ACN)

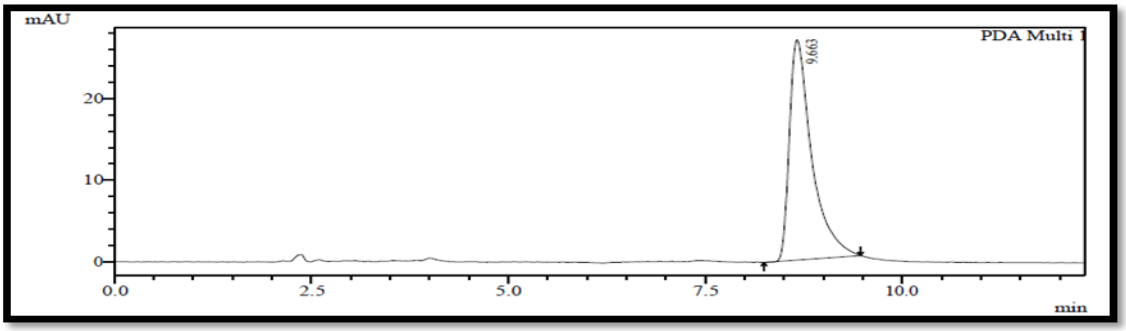


Figure 6. Chromatogram of AMI

Table 2. Linearity data of AMI and PGB

Parameter	AMI	PGB
Linearity range	4-14 µg/mL	30-105 µg/mL
Regression equation	y = 34259x + 214358	y = 557.229x + 6500
Avg of slope	34260	556.6
SD of intercept	6164.54	507.39
r^2	0.9984	0.9977

The LOQ of PGB and AMI were found to be 9.11 mcg/mL and 1.79 mcg/mL, respectively. LOD was found to be 3.00 mcg/mL and 0.59 mcg/mL, respectively. The result of LOD and LOQ are summarized in Table 5.

Table 3. Repeatability data of AMI and PGB

Drug	Concentration (µg/mL)	Mean concentration found (µg/mL) ± SD (n = 6)	% RSD
AMI	6	5.97 ± 0.03	0.63
PGB	45	45.36 ± 0.37	0.83

Table 4. Data of Intraday and Interday Precision of AMI and PGB

Drug	Concentration (µg/mL)	Intraday precision		Interday precision	
		Mean concentration found (µg/mL) ± SD (n=3)	% RSD	Mean concentration found (µg/mL) ± SD (n=3)	% RSD
AMI	4.8	4.85 ± 0.02	0.43	4.98 ± 0.04	0.97
	6	5.97 ± 0.06	0.62	6.34 ± 0.04	0.42
	7.2	7.23 ± 0.05	0.38	7.09 ± 0.13	0.87
PGB	36	36.50 ± 0.75	0.93	36.68 ± 0.93	1.26
	42	42.38 ± 1.63	1.02	42.90 ± 1.06	0.68
	54	54.59 ± 1.41	0.59	54.32 ± 2.56	1.07

Table 5. LOD and LOQ

Parameter	AMI	PGB
LOD (µg/ml)	0.59	3.00
LOQ (µg/ml)	1.79	9.11

The method accuracy was proven by the recovery test. A known amount of AMI standard and PGB standard were added to aliquots of the sample solution and, then, diluted to yield total concentrations as 10.8, 12, and 13.2 mcg/mL for AMI and 81, 90, and 99 mcg/mL for PGB. The resultant % recovery was found to be 99.79–100.95% for AMI and 99.64–101.87% for PGB with % RSD of < 2%. The result of accuracy is summarized in Table 6.

Table 6. Accuracy data for AMI and PGB

Amitriptyline Hydrochloride				
Recovery level (%)	Amount taken (µg/mL)	Amount added (µg/mL)	% Mean recovery ± SD (n=3)	%RSD
80	6	4.8	99.79 ± 0.47	0.47
100	6	6	100.95 ± 0.21	0.21
120	6	7.2	100.07 ± 0.73	0.73
Pregabalin				
Recovery level (%)	Amount taken (µg/mL)	Amount added (µg/mL)	% Mean recovery ± SD (n=3)	%RSD
80	45	36	99.64 ± 0.55	0.55
100	45	45	101.30 ± 1.24	1.24
120	45	54	101.87 ± 0.37	0.37

The %RSD value of assay determined for the same sample under original conditions and robustness conditions was less than 2%, indicating that the developed method was robust. The result of robustness is summarized in Table 7.

Table 7. Robustness data for AMI and PGB

Sr. No.	Parameter	AMI			PGB		
		Normal condition	Variable 1	Variable 2	Normal condition	Variable 1	Variable 2
1	pH	4.0	3.9	4.1	4.0	3.9	4.1
	Area (Mean)	561270	659286	717057	51986	44288	57544
	SD	1959.74	9942.76	12720.67	856.18	6243.05	9452.33
	% RSD	0.34	1.5	1.7	1.6	1.4	1.6
2	flow rate	1.0 mL/min	0.9 mL/min	1.1 mL/min	1.0 mL/min	0.9 mL/min	1.1 mL/min
	Area (Mean)	561270	862080	626360	51986	57637	40903
	SD	1959.74	9125.04	6722.29	856.18	1057.69	706.67
	% RSD	0.34	1.0	1.07	1.6	1.83	1.7
3	mobile phase (ACN:Buffer)	60:40	58:42	62:38	60:40	58:42	62:38
	Area (Mean)	561270	716286	652012	51986	61388	55349
	SD	1959.74	8041.55	10422.15	856.18	695.85	673.44
	% RSD	0.34	1.1	1.5	1.6	1.1	1.2

Forced Degradation studies in Formulation

Acid degradation studies showed the presence of an additional peak at 5.3 minutes in the chromatogram of acid degradation. This extra peak represents the formation of degradation products. The chromatogram for acidic degradation of AMI and PGB showed degradation of approximately 14.27% and 13.16%, respectively (Figure 10). However, acid degradation is lesser as compared to alkali. Alkali degradation studies showed the presence of additional peaks at 3.2 minutes in the chromatogram of Basic chromatogram. This extra peak represents the formation of degradation products. The chromatogram for basic degradation of the actives showed degradation of approximately 12.89% and 15.91% for AMI and PGB, respectively (Figure 11). In oxidative degradation AMI and PGB were evidenced at 18.57% and 12.49%, respectively, in presence to oxidation degradation (Figure 12). The AMI and PGB were degraded 3.66% and 1.80 % under neutral condition (Figure 13). Photo drgradation resulted in degradation of 12.30 % and 27.39% for AMI and PGB, respectively (Figure 14). The chromatograms of AMI and PGB showed 7.80% and 23.55% degradation, respectively under thermal condition (Figure 13). The % degradation of AMI and PGB under various stress conditions are summarised in Table 8.

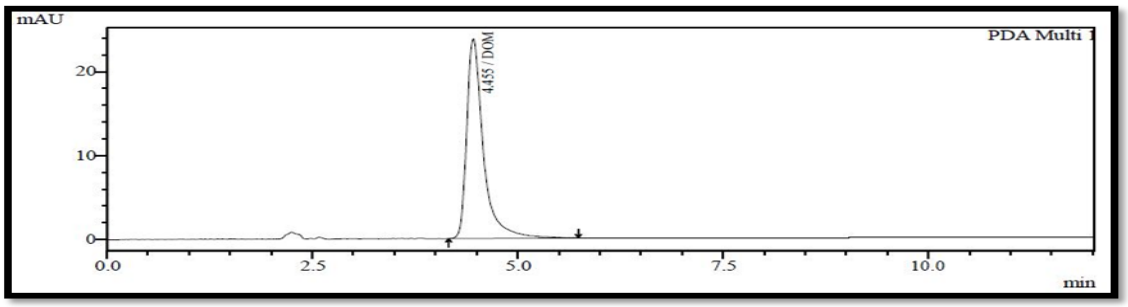


Figure 7. Chromatogram of PGB

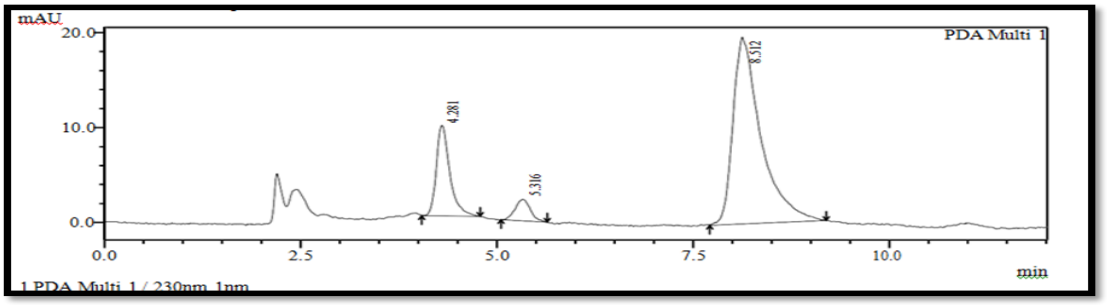


Figure 8. Chroamtogram of acid degradation

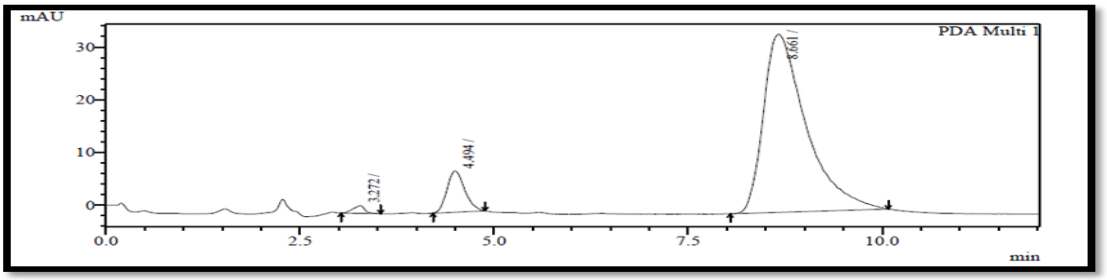


Figure 9. Chroamtogram of Base degradation

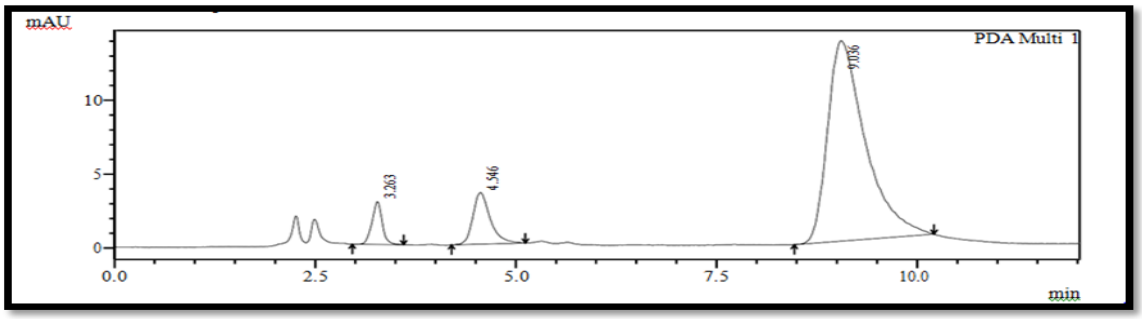


Figure 10. Chroamtogram of Oxidative degradation

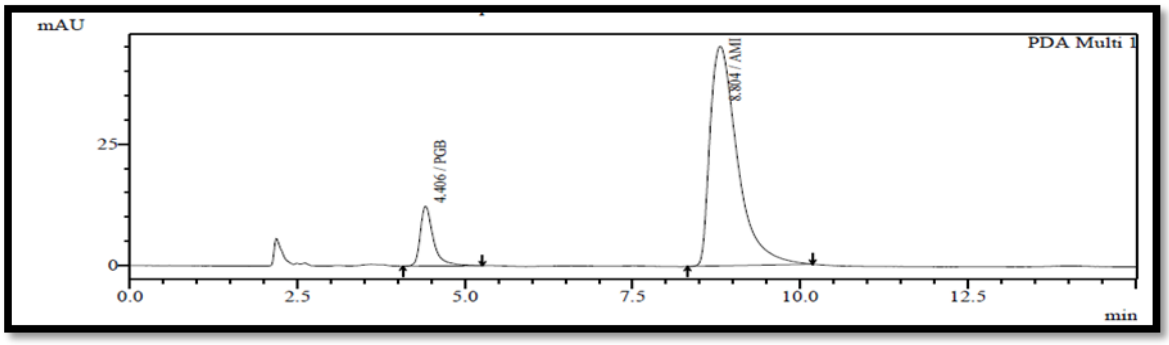


Figure 11. Chroamtogram of Neutral degradation

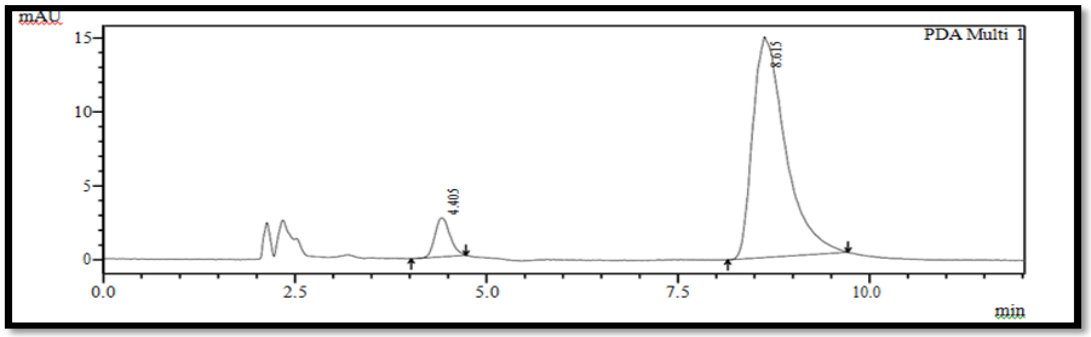


Figure 12. Chroamtogram of photo degradation

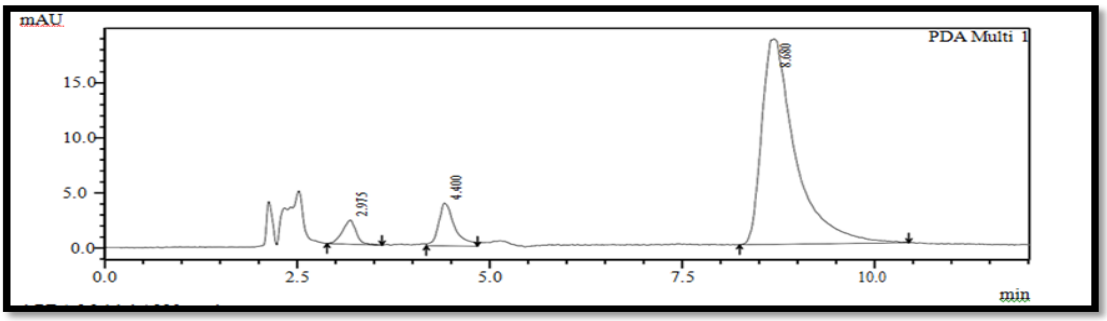


Figure 13. Chroamtogram of thermal degradation

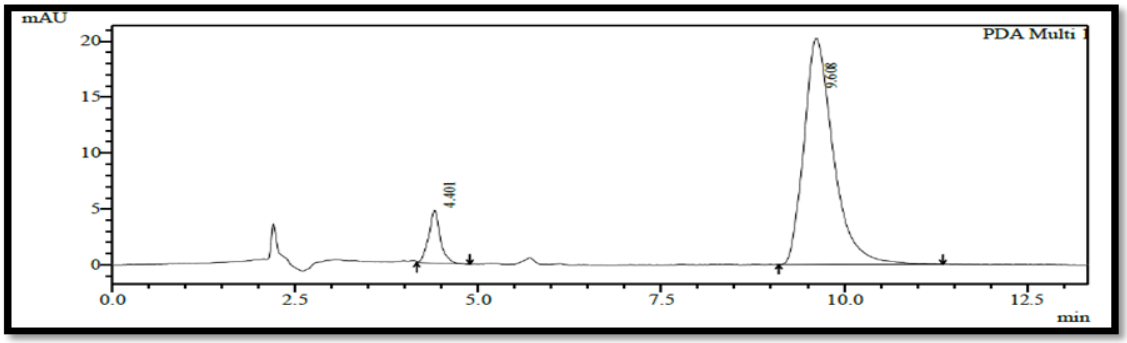


Figure 14. Chromatogram of sample formulation

Analysis of Commercial Formulation

The proposed method was applied to the determination of AMI and PGB in its tablet dosage form and the result of these assays yielded 99.58% and 99.16% for AMI and PGB, respectively, (RSD is <2%). The result of the assay (mentioned in table) indicates that the method is selective for the assay of AMI and PGB without interference from the excipients used in these tablets. Chromatogram of sample formulation is shown in Figure 14. and results of Assay is summarized in Table 9.

Table 8. Summary of degradation studies of AMI and PGB using proposed HPLC method

Degradation Condition	%Degradation		Peak purity index	
	AMI	PGB	AMI	PGB
Acid Degradation (0.1 N HCl, 4 Hrs.)	14.27%	13.16%	0.994	0.981
Base Degradation (0.1 N NaOH, 2 Hrs.)	12.89%	15.91%	0.991	0.987
H_2O_2 Degradation (3 % H_2O_2, 2 Hrs.)	18.57%	12.49%	0.984	0.988
Thermal Degradation (105 °C, 24 Hrs.)	7.80%	23.55%	0.993	0.989
Photolytic Degradation (24 Hrs.)	12.30%	27.39%	0.989	0.996

Table 9. Assay

Drug	Label claim (mg)	%Assay ± SD (n=6)
AMI	10	99.58 ± 0.55
PGB	75	99.16 ± 0.49

Conclusions

In this study, a simple, specific, accurate, precise and reliable isocratic stability indicating HPLC-DAD procedure was developed for the Assay of AMI and PGB in their pharmaceutical combination. The two analytes were subjected to forced degradation using several stress (neutral, acid/base, oxidative, photolytic and thermal) conditions and the proposed method was successfully employed for the resolution of the analytes peaks from those of the forced degradation products. The most labile condition for AMI was oxidative and the most labile condition for PGB was basic. Range of % degradation in forced degradation was 5-30% which was followed here. The developed method made use of DAD as a tool for peak identify & purity conformation. Finally, the method was thoroughly validated and, therefore, it was recommended for routine analysis and for checking quality during stability studies of the cited drugs.

References

[1] Blessy M., Patel R.D., Prajapati P.N., Agrawal Y.K. *J. Pharm. Anal.,* 2014, **4**:159

[2] Singh S., Bakshi M. *Pharm. Technol*., 2000, **1**:14

[3] ICH Q1B, Stability testing: photo stability testing new drug substances and products :Geneva, 1996

[4] Ritter J., Flower R., Henderson G., Rang H., Rang & Dales's Pharmacology; 8th ed. Elseveir publication. 2012, pp. 582 – 587

[5] Singh R., Indian Pharmacopoeia, Ghaziabad, 2014, Volume-II, pp 1043 – 1044 & 2548

[6] Patel N., Kaur G., Patel M., Sakhareliya B., Bhumika B. *J. Pharm. Sci. Bioscient. Res.*, 2016, **6**:547

[7] Sneha P., Srinivas P. *Indo Am. J. Pharm. Sci.,* 2015, **2**:1038

[8] Nagaraju KS, Rao BS, Kiran BV, Nagarjuna A. *Int. J. Pharm. Sci. Res.,* 2013, **4**:2782

[9] Douša M., Gibala P., Lemr K. J. Pharm. Biomed. Anal., 2010, 53:717

[10] Reddy M.C., Sekhar K.B. Int. J. Chem. Pharm. Sci., 2012, 3:40

[11] Oh J.S., Lim S.H., Ryu S.H., Kim K.H., Lee K.S., Song W.H., Park J.S. *Asian J. Pharm. Sci.,* 2016, **11**:229

[12] Vijaya Bhaskar Reddy T., Ramu G., Ravindra babu N., Rambabu C. *J. Pharm. Res.,* 2012, **5**:3896

[13] Farag R.S., Mahmoud A. *Asi. J. Res. Chem.,* 2011, **4**:24

[14] Jaswanth B.S., Kumar R.V., Sinha V.R. An Indian Journal, 2013, 12:377

[15] Önal A., Sagirli O. *Spectrochim. Acta - Part A Mol. Biomol. Spectrosc.*, 2009, **72**:68

[16] Patil D.D., Patil M.S., Wani Y.B. *J. Assoc. Arab Univ. Basic Appl. Sci.,* 2016, **21**:31

[17] Gor J.H., Jain H.K., Gujar K.N. *Int. J. Drug Res.,* 2013, **5**:356

[18] Vukkum P., Mosesbabu J., Murlikrishnan R. *Int. J. Pharm. Sci. Res.,* 2015, **6**:2241

[19] Souri E, Eskandari M, Barazandeh T, Adib N, Ahmadkhaniha R. *Asian J. Chem.,* 2013, **25**:7332

[20] Kasawar G.B., Farooqui M.N. *Int. J. Pharm. Sci. Res.,* 2010,**72**:517

[21] Karavadi T.M., Challa B.R. *J. Liq. Chromatog. Relat. Technol.,* 2014, **37**:130

[22] Berry D., Millington C. Ther. Drug Monit., 2005, **27**:451

[23] Shen Y, Zhu RH, Li H De, Liu YW, Xu P. *J. Pharm. Biomed. Anal.,* 2010, **53**:735

[24] Rabie S.F., Mahmoud Z.D., Wael M.F., Hamdi A.H. *Int. J. Chem. Anal. Sci.,* 2013, **4**:120

[25] ICH Q2(R1), Validation of Analytical Procedures : Geneva, 2005.

[26] George N., Forced Degradation as an Integral Part of HPLC Stability-Indicating Method Development: 2010

[27] Snyder L. R., Kirkland J.J., Glajch J.L. Practical HPLC Method Development. 2nd ed, John Wiley & sons Ltd, 1997

How to cite this manuscript: Amit J. Vyas, Grishma P. Nathwani*, Ajay I. Patel, Nilesh K. Patel, Ashok B. Patel Validated Stability Indicating RP-HPLC DAD Method for Simultaneous Determination of Amitriptyline Hydrochloride and Pregabalin in Presence of Stress degradation products in tablet dosage form. Chemical Methodologies 3(4), 2019, 494-508. DOI: 10.22034/chemm.2019.154554.1107.