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A New Stability-Indicating RP-HPLC Method for the Determination of Retigabine in Oral Dosage Form

K. Swapna and K. Ravindhranath*

Department of Chemistry, Bapatla College of Engineering, Bapatla, A.P, **INDIA**

Email: ravindhranath.kunta@gmail.com

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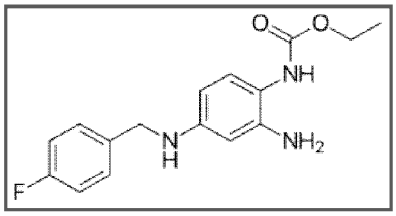
ABSTRACT

A new stability-indicating RP-HPLC method was developed for the determination of retigabine in oral dosage form, using a ODS, C_{18} RP-Column (Make: 250 mmx4.6 mm I.D; particle size 5µm) and a mobile phase composed of phosphate buffer (pH-3.8) and acetonitrile in the ratio of 55:45 %v/v at a flow rate of 1.0mL min^{-1}. The retention time of retigabine was found to be 2.741 min, respectively. Linearity was established for retigabine in the range of 10-60µg/ml, respectively. Retigabine was subjected to acid and base hydrolysis, oxidation and photolytic degradation conditions and the degradation products of retigabine were well resolved from the pure drug. This method can be successfully employed for the quantitative analysis of retigabine in various formulations respectively.

Graphical Abstract



Molecular structure of Retigabine

Keywords: Retigabine, Stability-indicating method.

INTRODUCTION

Retigabine [**1-5**] N-[2-amino-4-(4-fluorobenzylamino) phenyl] carbamic acid ethyl ester is a novel, anticonvulsant drug approved for use in partial-onset seizures. It works primarily as a potassium channel opener, by activating a certain family of voltage-gated potassium channels in the brain (Fig.1).

Five HPLC methods [**6-9**] and one stability-indicating RP-HPLC method [**10**] have been reported for the determination of retigabine in pharmaceutical dosage forms. This enabled the author to make an attempt in developing a new stability indicating RP-HPLC assay method for retigabine in

pharmaceutical dosage forms under various stress condition like acid hydrolysis, base hydrolysis, oxidation and photolytic stress.

The present paper describes the development and validation of a stability-indicating liquid chromatographic analytical method for retigabine in pure and in tablet formulations.

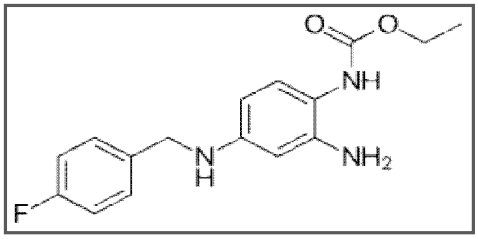


Figure 1. Molecular structure of Retigabine

MATERIALS AND METHODS

Instrumentation: The present analysis was performed using HPLC system (Waters Alliance 2695 separations module) equipped with 600e controller pump, 776 auto sampler, 2487 dual variable wavelength UV detector. The data was recorded and analyzed with Empower software on Dell computer. A stainless steel ODS, C_{18} RP-Column (4.6mm x250mm) purchased from Waters Corporation (Bedford, MA, USA) was used in the present assay. Degassing of the mobile phase was done using a Loba ultrasonic bath sonicator. Dona analytical balance was used for weighing the materials.

Chemicals and Reagents: Retigabine (99.9% pure API) was obtained as a gift sample from Angle Biopharma Labs, Ahmadabad. Its dosage formulation in the brand name of Trobalt tablets (Each 100mg tablet equivalent to 100mg of retigabine) was purchased from local pharmacy. Potassium dihydrogen phosphate (AR grade), dipotassium hydrogen phosphate (AR grade), orthophosphoric acid (AR grade), methanol (HPLC grade) and Acetonitrile (HPLC grade) were purchased from Merck, Mumbai. Milli-Q water was used for preparing buffer and other reagent solutions respectively.

Preparation of Buffer Solution: 1.625g of potassium dihydrogen phosphate and 0.8g of dipotassium hydrogen phosphate were weighted and dissolved in 1000 mL HPLC grade water and then adjust to pH 3.8 with orthophosphoric acid. This buffer solution was filtered and degassed prior to the assay.

Preparation of Mobile Phase: The mobile phase was prepared by dissolving phosphate buffer (pH-3.8) and Acetonitrile in the ratio of 55:45 %v/v. Prior to the assay the mobile phase is filtered and degassed.

Preparation of Diluent: Methanol is used as diluent in the present assay.

Preparation of Standard Stock Solution: Standard stock solution of retigabine was prepared by transferring accurately weighing 25 mg of retigabine (99.9% pure API) into a 25 mL volumetric flask containing 10 mL of methanol. Mix the contents and make up to volume with the same diluent. This stock solution was stored at 2-8°C and was protected from light. From standard stock solution working standard solutions of retigabine in the concentration range of 10 to 60 µg mL^{-1} were prepared by diluting aliquots of stock solution of retigabine in 10 mL volumetric flasks with the mobile phase. All the above volumetric flasks of working standard solutions were wrapped with aluminum foil and were protected from light. Prior to injection the working standard solutions were filtered through a 0.45µm membrane filter respectively.

Preparation of Sample Solution: Ten oral tablets of Trobalt (Label claim 100 mg of retigabine) purchased from local pharmacy were weighed accurately and grinded to fine powder. The powder equivalent to 100 mg of retigabine sample is transferred into a 100mL volumetric flask containing 50 mL of diluent, Sonicate the contents to dissolve. Degas the solution and make up to the volume by the diluent. Further, dilutions were made to obtain solutions of final concentration within the linearity range, and the procedure previously described for working standard solution was followed respectively.

Chromatographic Conditions: ODS, C_{18} RP-Column (Make: 250 mmx4.6 mm I.D; particle size 5 µm) was used for analysis at ambient column temperature. The mobile phase comprising of phosphate buffer (pH-3.8) and Acetonitrile in the ratio of 55:45 %v/v was pumped through the column at a flow rate of 1.0 mL min^{-1}. The sample injection volume was 10 µL. The photodiode array detector was set to a wavelength of 240 nm for the detection and Chromatographic runtime was 6 min.

RESULTS AND DISCUSSION

Method Development: In the present study a systematic study of the effect of various factors [i.e, the influence of column, aqueous and organic phase for mobile phase, mobile phase proportion, wavelength, diluents, concentration of analyte and other chromatographic parameters] involved development of new method was carried out by varying one parameter at a time and keeping all other conditions constant.

From these studies it was revealed that in the current study ODS, C_{18} RP-Column (4.6 mm x 250 mm) having 5µm particle size was used among the other columns because of its advantages of high degree of retention, high resolution capacity, better reproducibility, ability to produce lower back pressure and low degree of tailing. A good symmetrical peak for retigabine was obtained, when water was replaced by phosphate buffer (adjusted to acidic pH by orthophosphoric acid) as aqueous phase in mobile phase. Preliminary trials on mobile phase proportion that the proportion of phosphate buffer (pH-3.8) and Acetonitrile in the ratio of 55:45 %v/v was finalized as it gave good symmetrical peak for retigabine respectively.

The appropriate wavelength for determination of retigabine was scanned by UV-visible spectrophotometer and was observed that the maximum absorbance (λ_{max}) was obtained at 240 nm. At this wavelength retigabine offered high response with good linearity. The best separation with adequate resolution and symmetric peak of retigabine was obtained when the injection volume was fixed to 10 µL with a flow rate was set to 1.0 mL min^{-1} for the mobile phase respectively.

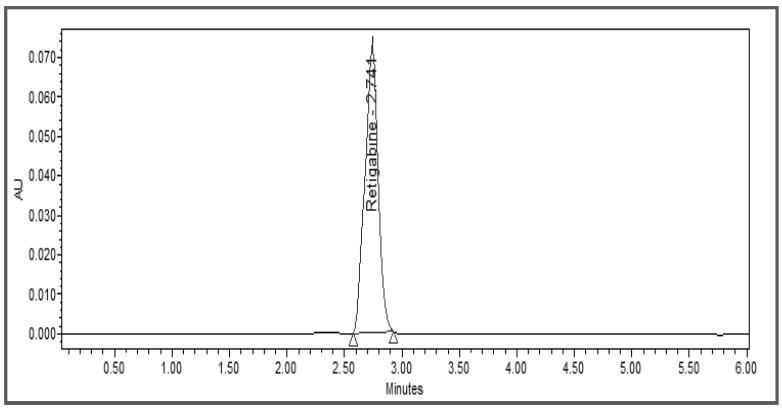


Figure 2. Optimized chromatogram of Retigabine.

On this finalized chromatographic conditions, obtained chromatogram of retigabine exhibited good peak symmetry with higher theoretical plates and elution time of 2.741 minutes respectively. The

representative chromatogram of retigabine is shown in figure 2.

Method Validation: After fixing the optimization studies the developed method was validated as per ICH guidelines which include system suitability, specificity, linearity, accuracy, precision, robustness, ruggedness, sensitivity, limit of detection and quantification.

System Suitability: Six consecutive injections equilibrated initially with the above said mobile phase were used to evaluate the system suitability of retigabine assay. The number of theoretical plates was higher than 2000, making the proposed method acceptable for the assay of retigabine in dosage forms as reported in table 1.

Table 1. System Suitability Parameters

PARAMETERS	RETIGABINE
Retention time	2.741
USP Plate count	6850
USP Tailing	1.129

Forced Degradation studies: In the present study, drug was applied to various stress conditions of degradation as per the recommended guidelines of ICH. The degradation samples were prepared by transferring powdered tablets, equivalent to 10mg of drug into a 250 mL round bottomed flask. Then drug content was employed for acidic, alkaline and oxidant media and also for photolytic stress conditions. After the degradation treatments were completed, the stress content solutions were allowed to equilibrate to room temperature and diluted with diluent to attain 100 µg mL^{-1} of retigabine concentrations. Specific degradation conditions were described as follows.

Acid Induced Degradation: Solutions containing 100 mg of Retigabine was treated with 10mL 1N HCl sonicate or reflux for 30minutes and then add 10 mL of 1N NaOH to neutralize and make up with methanol (diluent).

Alkaline Degradation: Solutions containing 100 mg of Retigabine was treated with 10 mL 1N NaOH sonicate or reflex for 30 min and then add 10 mL of 1N HCL to neutralize and make up with methanol (diluent).

Oxidative condition: Solution containing 100 mg of Retigabine was treated with 6% w/v H_2O_2 at 40°C for 6 hrs was cooled and diluted with methanol.

Photolytic degradation study: 100 mg of Retigabine was exposed to the UV light/sunlight for 7 days. This drug powder was transferred in 50 mL volumetric flask, diluted to the volume with methanol.

The chromatograms of retigabine under acidic, basic, oxidative and photolytic stress conditions (Fig.3a-d) revealed that retigabine was found to be more stable and did not showed any degradation and is eluted from the column respectively. From the above stress studies, it is revealed that retigabine was not fully degraded. In acidic degradation, it was found that around 9.0% of the drug was degraded. In alkali degradation, around 6.0% of the drug degraded. And the degradation found in oxidative condition was up to 10%. In photolytic degradation, it was found that around 3.0% of the drug degraded respectively. From these studies (Table 2) it was concluded that the degradation products did not interfere in the detection analysis of retigabine establishing the high stability of the developed method.

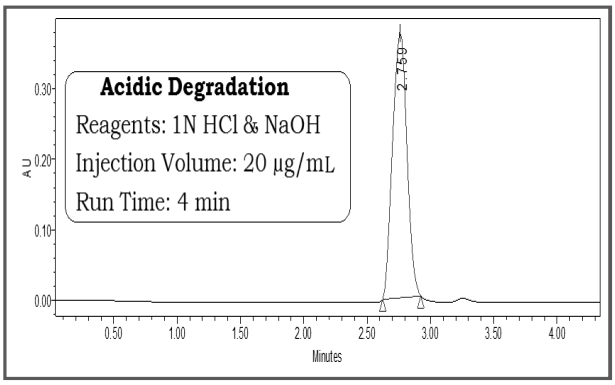


Figure 3a. Chromatogram of retigabine in acid stress

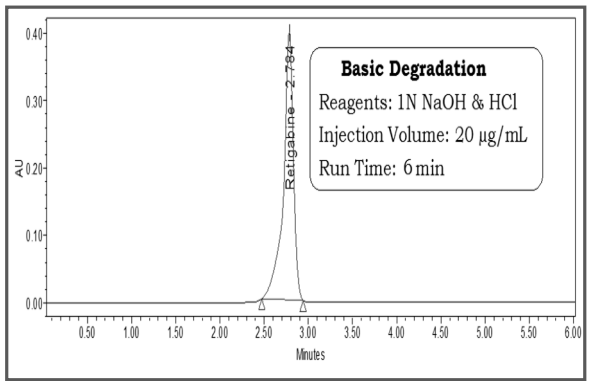


Figure 3b. Chromatogram of retigabine in base stress

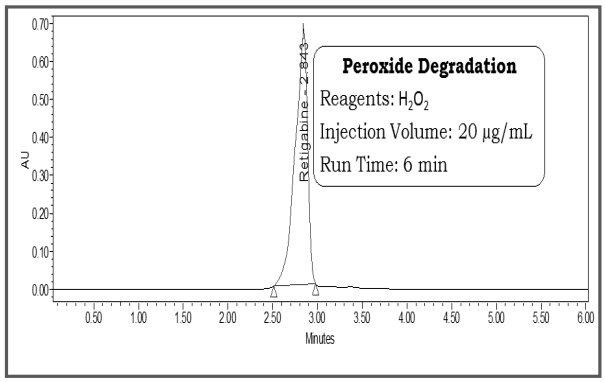


Figure 3c. Chromatogram of retigabine in oxidative stress

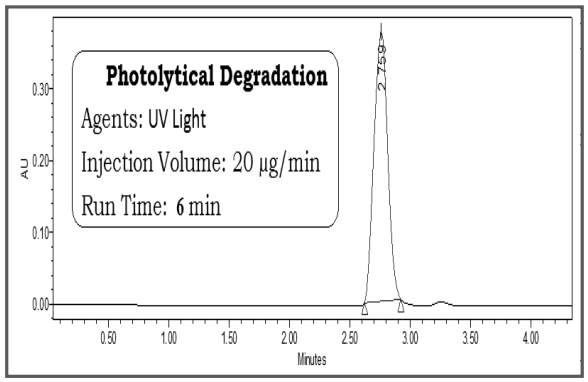


Figure 3d. Chromatogram of retigabine in photolytic stress

Table 2. Forced Degradation Results of Retigabine In Various Stress Conditions

Stress Conditions	Drug recovered (%)	Drug decomposed (%)
Standard Drug	100	0
Acidic degradation	91	9.0
Basic degradation	94	6.0
Oxidative degradation	90	10
Photolytic degradation	97	3.0

Linearity: For linearity studies concentration levels of retigabine corresponding to 50, 75, 100, 12 and 150% of test solution were prepared separately. 10 µL of each concentration was injected into the HPLC system and the response factor was read at 240 nm. The respective chromatogram corresponding to each concentration was recorded**.**

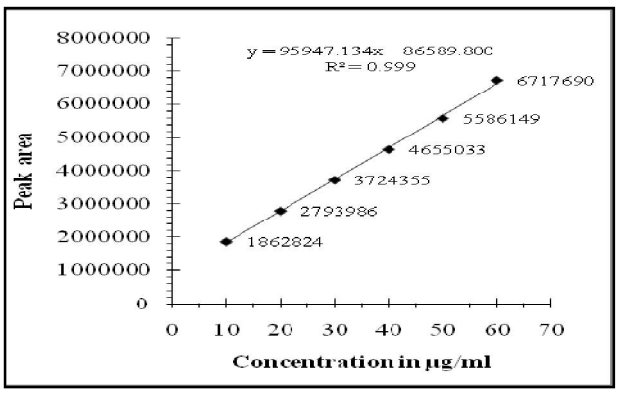


Figure 4. Linearity curve of retigabine

Table 3. Linearity Performance Calculations for Retigabine

Concentration in % Level	Concentration in µg mL^{-1}	Area in mAU
25	10	1862824
50	20	2793986
75	30	3724355
100	40	4655033
125	50	5586149
150	60	6717690
Regression equation; Intercept (a)		865189.8
Slope (b)		95947.134
Correlation coefficient		0.9994
Standard deviation on intercept (S_a)		1657.09738
Standard deviation on slope (S_b)		64534.6713
LOD(µg mL^{-1})		2.01
LOQ(µg mL^{-1})		6.72

Limit of Detection and Limit of Quantification: The LOD and LOQ value of retigabine by the proposed method was found to be 2.01µg mL^{-1} and 6.72 µg mL^{-1} respectively.

Precision: In this accord six replicate injections one fixed standard concentration (40 µg mL^{-1} or 100% conc. level) of retigabine solution were injected and the percent relative standard deviation (% RSD) was calculated. It was found that the %RSD values are well within the acceptable criteria (< 2.0). Results of system precision studies are shown in table 4 respectively.

Table 4. Precision Of The Proposed RP-HPLC Method

Injection Number	Name of the Drug and Concentration(100 µg mL^{-1})	Retention time (min)	Peak Area
1	Retigabine	2.750	4691321
2	Retigabine	2.761	4681212
3	Retigabine	2.754	4661224
4	Retigabine	2.764	4571231
5	Retigabine	2.702	4691224
6	Retigabine	2.704	4591674
*Mean		2.739	4647891
*Standard Deviation		0.028	53083.01
*% RSD		1.03	1.14

Accuracy: The accuracy of the present proposed method was assessed by recovery studies at three concentrations (corresponding to 50,100,150% of test solution concentration) of retigabine covering within the linearity range. Each concentration, were analyzed in triplicate at each level as per the proposed method and the percent recovery and % RSD was calculated and results are compiled in table 5. These results indicated a high degree of accuracy of the proposed method was obtained for the assay of retigabine.

Table 5. Recovery Studies of The Proposed RP-HPLC Method

S.No	50 % AREA	100 % AREA	150 % AREA
Injection-1	275392	466964	672247
Injection-2	274356	465784	674549
Injection-3	277454	467433	677865
Avg *	275734	466727	674887
Amt Recovered*	19.98	39.96	59.94
%Recovery*	99.80	99.80	99.90

*Average of three determinations

Ruggedness: The ruggedness of the present RP-HPLC method was determined by carrying out the experiment by different analysts using different columns of similar types. The percentage %RSD of different preparations assay values with two different analysts were 1.14 and 1.00 respectively revealing the proposed method is rugged (Table 6).

Table 6. Ruggedness Studies of Proviverrine HCl with the Proposed Method

S.No	Analyst-1	Analyst -2
	Area	Area
Injection-1	4691321	4672634
Injection-2	4681212	4656312
Injection-3	4661224	4550506
Injection-4	4571231	4654397
Injection-5	4691224	4675868
Injection-6	4591674	4632851
Avg*	4647891	4640428
Std Dev*	53083.01	46656.26
% RSD*	1.14	1.00

*Average of three determinations; SD=Standard Deviation; %RSD= Percentage Relative standard deviation

Robustness: Robustness of the present assay method was determined by small deliberate changes in flow rate, and temperature. It was observed that there were no marked changes in chromatograms, which demonstrated that the developed RP-HPLC method is robust and is represented in table 7.

Table 7. Evaluation Data of Robustness Study

S.No	Parameter	Plate count	Tailing Factor
1	Flow rate (0.9 mL min^{-1})	9005.35	1.300
2	*Flow rate (1.0 mL min^{-1})	9461.75	1.360
3	Flow rate (1.2 mL min^{-1})	9707.025	1.180
4	Column temperature (20ºC)	9739	1.300
5	*Column temperature (25ºC)	9961	1.360
6	Column temperature (30ºC)	9875	1.322

*Results for actual flow (1.0 mL min^{-1}) and column temperature (25ºC) used in the present assay

APPLICATION

Assay of retigabine in tablet dosage form: The proposed RP-HPLC method was applied for the estimation of retigabine in tablet dosage form Trobalt (Label claim 100 mg of retigabine). The results are shown in table 8. The high recovery (99.91%) value confirmed the appropriateness of the proposed RP-HPLC method for the routine analysis of retigabine in tablet dosage forms.

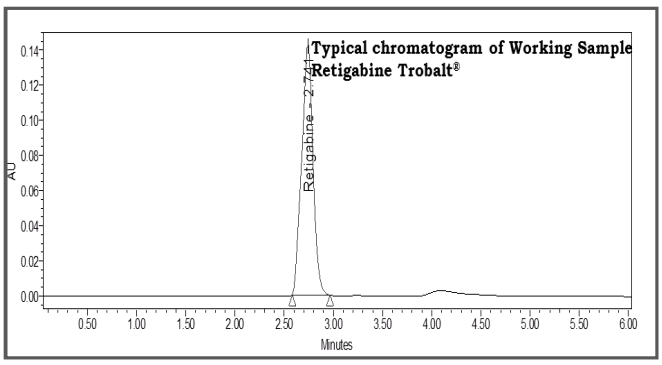


Figure 5. Chromatogram of retigabine in formulation

Table 8. Results of Analysis of Tablet Containing Retigabine

Formulation Name	Standard Peak area	Sample Peak area	Labeled Amount (mg)	Amount Found(mg)	*%Assay ±RSD
Trobalt	4655033	4691424	100	99.91	99.91±0.14

* Average of three determinations

CONCLUSION

A new simple, precise, stability indicating RP-HPLC assay method was developed for retigabine in bulk and pharmaceutical dosage form. It has been proved that the method is selective and linear between concentration ranges of 10 – 60 µg mL^{-1} for retigabine. The LOD was found to be 2.01 µg mL^{-1} and LOQ was found to be 6.72 µg mL^{-1} for retigabine. The proposed method was also found to be accurate and precise, as indicated by recovery studies close to 100 and %RSD is not more than 2. Statistical analysis proved that the method is suitable for the analysis of retigabine in pharmaceutical formulation without any interference from the excipients that is recommended for routine and quality-control analysis.

ACKNOWLEDGEMENTS

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