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Development and Validation of New UV-Spectrophotometric Method for The Quantitation of Retigabine In Pure and Formulations

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Abstract:*A simple, accurate, precise, economical and reproducible UV-spectrophotometric method has been developed for the simultaneous estimation of Retigabine in bulk and in combined tablet dosage form. The stock solutions were prepared in methanol followed by the further required dilutions with distilled water. This method involves the formation and solving of simultaneous equations at 254 nm as absorbance maxima of Retigabine respectively. Linearity obeyed the concentration range of 25µg/mL to 75µg/mL for Retigabine respectively. The results of analysis were validated statistically and by recovery studies. The % RSD for the recovery study was less than 2.0. The proposed method can be effectively applied for the estimation of Retigabine in bulk and in combined tablet dosage form*

Keywords: *UV-Spectrophotometric, Retigabine, Validation*

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I. Introduction

Retigabine or Ezogabine is an anticonvulsant used as an adjunctive treatment for partial epilepsies in treatment-experienced adult patients. Retigabine works primarily as a potassium channel opener that is, by activating a certain family of voltage-gated potassium channels in the brain. This mechanism of action is unique among antiepileptic drugs, and may hold promise for the treatment of other neurologic conditions, including tinnitus, migraine and neuropathic pain.
IUPAC name: Ethyl *N*-[2-amino-4-[(4-fluorophenyl)methylamino]phenyl]carbamate.

BCS Classification: BCS Class II.
Molecular Formula: $C_{16}H_{18}FN_3O_2$
Molecular Weight: 303.3 g/mol
Color: White to slightly colored crystalline powder
Solubility: At room temperature it is practically insoluble in aqueous media above pH 4.0, while the solubility is higher in polar organic solvents.

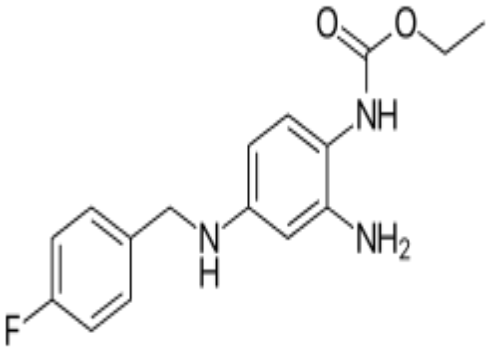

Figure 1.1: Chemical structure of Retigabine

Literature survey carried out revealed that several methods have been reported for estimation of Retigabine by using, UV Spectrophotometric method [1-2], RP-HPLC Methods [3-10] are available to determine Retigabine in tablet dosage form. Although reports are available on stability indicating HPLC methods, the information provided is incomplete as well as results are contrast. Basing on this the author attempted to develop a simple, inexpensive UV-spectrophotometric method for the determination of retigabine in pure and in dosage forms. The method proposed by the author is less tedious and economical.

II. Experimental

Chemicals and Reagents: Retigabine (99.9% Pure) was supplied by Dr Reddys Labs, Hyderabad. and its formulation (Tablets) in the brand name of Trobalt: 100mg of Retigabine were purchased from local pharmacy. Methanol of analytic grade was purchased from Merck and was used for the preparation of standard and sample solutions without further purification.

Instrumentation: Shimadzu UV/Vis Spectrophotometer (Model-2450) equipped with UV probe software was used in the present assay. For dilutions various micropipettes of volumes 10-100µL were used. All weighing experiments were done on Shimadzu Digital Analytical Balance (Japan) and standard glass ware (class-A) was used for preparing of solution.

Diluent preparation: Methanol of analytical grade was used as diluent.

Standard preparation:

Accurately weigh 100mg of retigabine test standard and transfer into a 100mL volumetric flask containing 25mL of methanol solvent. This was sonicated for about 5 min to dissolve it and the resultant solution was further diluted to 100mL with methanol solvent (Diluent). Working standard solutions in concentration range of 25 - 75µg/mL were prepared by transferring aliquot of the above stock solution with micropipette to a series of different 100mL and diluted to the mark with the same diluent.

Sample preparation:

Taken 10 tablets of retigabine transfer in to mortar grinded to fine powder and was weighed. Then powder equivalent to 100mg was transferred into a 100mL clean dry volumetric flask, 70mL of diluent was added to it and was shaken by mechanical stirrer and sonicated for about 30 minutes by shaking at intervals of five minutes and was latter diluted up to the mark with diluent. Suitable aliquots of this solution was taken and diluted into a series of 10mL volumetric flask with the same diluent up to the mark, to obtain concentrations that obey within the beers law limit for the spectrophotometric measurement of retigabine according to the recommended procedure.

III. Results And Discussion

Method development

Working standard solution (50µg/mL) of retigabine prepared was subjected to scanning between 200–400 nm and the absorption maximum was determined and an optimal response was obtained at 254nm. This wavelength of 254nm was used for the quantification of standard and in dosage forms of retigabine respectively. The absorption spectrum so obtained was shown in Figure.1.2.

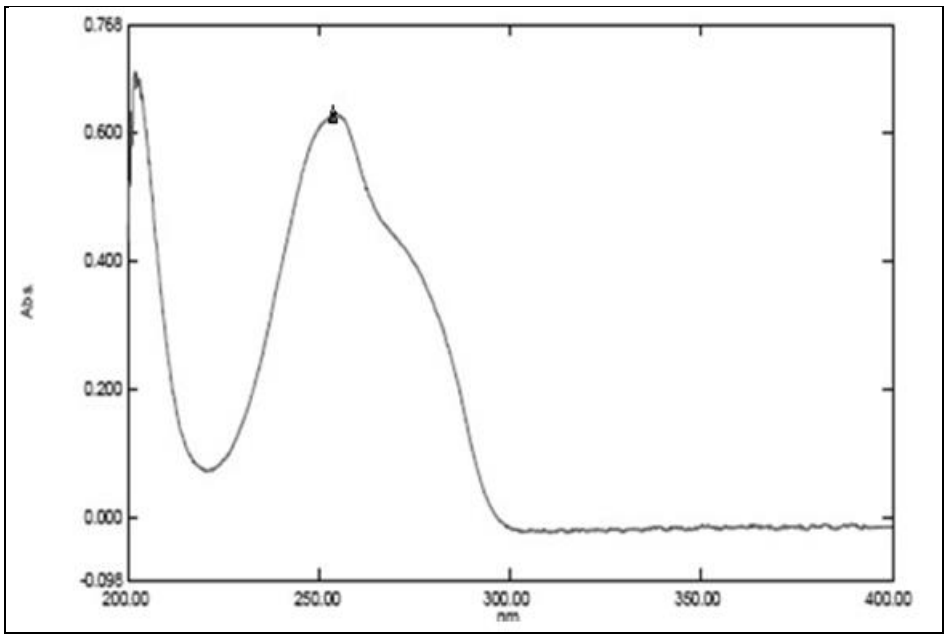

Figure 1.2: - A Typical UV Spectra of Retigabine

IV. Method validation

The developed UV spectrophotometric method extensively validated for assay of Retigabine using the following parameters.

4.1 Specificity

Preparation of blank solution:

The interference of blank at the working wavelength was scanned from 200-800nm and was observed the non-interference of blank at the working wavelength of 254nm for retigabine, revealing the specificity of the proposed UV spectrophotometric method for retigabine.

4.2 Method precision:

The precision of test method was evaluated by doing assay for six samples of Retigabine tablet as per test method. The content in mg and % label claim for Retigabine for each of the test preparation was calculated. The average content of the six preparations and % RSD for the six observations were calculated. The data were shown in Table: 1.1

Table: 1.1 Method precision data for Retigabine

S.No.	No. of Preparations	Absorbance
1	Preparation-1	0.616
2	Preparation-2	0.615
3	Preparation-3	0.613
4	Preparation-4	0.617
5	Preparation-5	0.618
6	Preparation-6	0.62
Avg.		0.617
STD Dev		0.0024
% RSD		0.39

4.3 Linearity of detector response

The linearity of an analytical method is its ability to obtain test results which has a definite mathematical relation to the concentration of analyte. The linearity of response for Retigabine was determined in the range of 50% to 150 % (25µg/ml to 75µg/ml for Retigabine). The calibration curve of analytical method was assessed by plotting concentration versus peak area and represented graphically. The correlation coefficient was found to be 0.9974.Therefore the UV method was found to be linear standard curve were calculated and given in **Figure: 1.3** to demonstrate the linearity of the proposed method. From the data obtained which given in **Table: 1.2** the method was found to be linear within the proposed range.

Table: 1.2 Linearity studies for Retigabine by proposed method

S.No	Retigabine		
	Linearity concentration (%)	**Concentration (µg/ml)**	**Average absorbance**
1	50	25.0	0.302
2	75	37.5	0.454
3	100	50.0	0.624
4	125	62.5	0.761
5	150	75.0	0.891
R^2			0.9974
Slope (m):			0.0119
Intercept (Y):			0.0124

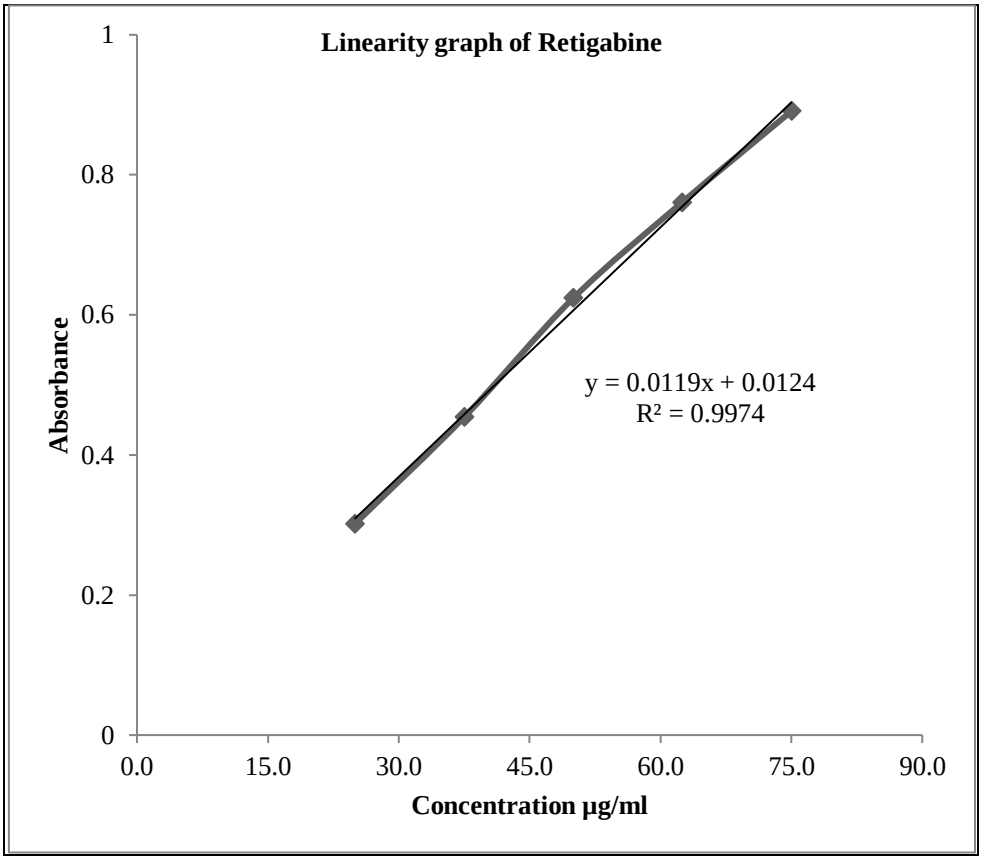

Figure: 1.3 Calibration curve for Retigabine

4.4 Accuracy

The accuracy of the method was determined on three concentration levels by recovery experiments. The recovery studies were carried out in triplicate preparations on composite blend collected from 20 tablets of Retigabine, analyzed as per the proposed method. The mean percentage recovery for 50%, 100%, 150% level was found to be 98.43, 98.97 and 98.03. They are within the acceptance limits. Therefore, the UV method for the determination of assay of Retigabine in formulation was found to be accurate. The data obtained which given in **Table: 1.3** the method was found to be accurate.

Table: 1.3 Recovery studies for Retigabine by proposed method

S.No	% Recovery results of Retigabine		
	50%	100%	150%
Preparation-1	0.304	0.613	0.904
Preparation-2	0.303	0.612	0.908
Preparation-3	0.302	0.611	0.907
Mean	0.303	0.611	0.906
%Recovery	98.43	98.97	98.03

4.4 Solution Stability:
In this study the absorbance of the same standard and sample solutions of retigabine in triplicate at intervals of 0 hours, 12hours, and 24 hours were recorded and the cumulative %recovery at each interval was determined. The % recoveries tabulated in **Table.1.4** revealed the stability of the proposed method.

Table 1.4: Results for solution stability of standard at room temperature

Time Interval	%Recovery	
(Hrs)	Standard(n=3)	Sample(n=3)
0	99.96	99.89
12	99.90	99.78
24	100.03	99.96

The standard and sample solutions are stable up to 24hrs at room temperature on bench top.

V. Conclusion

A new simple and validated UV-spectrophotometric method was developed for the assay of retigabine in pure and pharmaceutical formulations. The developed UV- spectrophotometric method exhibited the linearity in the range 25-75µg/ml respectively. The precision is exemplified by relative standard deviation of 0.393%. The percentage mean recovery was found to be in the range of 98.39-99.34%, in accuracy studies. The method was validated in accordance with ICH guidelines[38] like acceptance criteria with respect to selectivity, precision, accuracy, linearity, recovery.

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