RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE ANALYISIS OF EZOGABINE IN PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT:

A simple, selective, linear, precise and accurate RP-HPLC method was developed and validated for assay of Ezogabine in tablet dosage form. Isocratic elution at a flow rate of 1ml min^{-1} was employed on a symmetry C18 column at ambient temperature. The mobile phase consisted of Methanol:Water:Acetonitrile 30:50:20 (v/v/v). The UV detection wavelength was at 248 nm. The retention time for Ezogabine was 7.33 mins. The method was validated as per the ICH guidelines. The proposed method can be successfully applied for the estimation of Ezogabine in pharmaceutical dosage forms.

Key words: EZOGABINE, HPLC, Linearity, Precision, Recovery, 248nm.

INTRODUCTION:

Ezogabine (figure 1) is an anticonvulsant used for the treatment of partial epilepsies. The drug was developed by Valeant Pharmaceuticals and GlaxoSmithKline. It was approved by the European Medicines Agency under the trade name Trobalt on March 28, 2011, and by the United States Food and Drug Administration (FDA), under the trade name Potiga, on June 10, 2011.

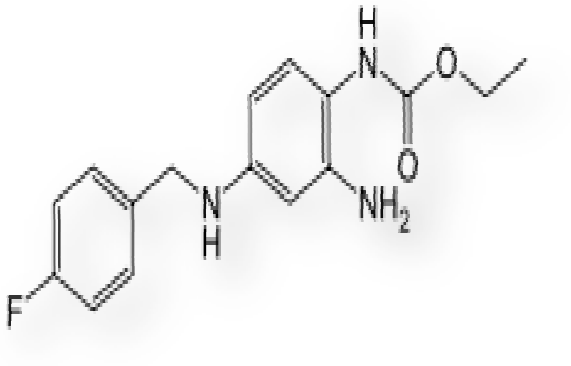

Figure 1: Structure of Ezogabine

EXPERIMENTAL:

Chemicals and reagents

All HPLC SOLVENTS used like Acetonitrile,Water and Methanol are of HPLC grade purchased from E.Merck,

Instrumentation and analytical conditions:

The analysis of the drug was carried out on Shimadzu HPLC model (VP series) containing LC-10AT (VP series) pump, variable wave length programmable UV/visible detector SPD-10AVP and rheodyne injector (7725i) with 20µl fixed loop. Chromatographic analysis was performed using Zodiac C-18 column with 250mm x 4.6mm internal diameter and 5µm particle size. Shimadzu electronic balance (AX-200) was used for weighing. Isocratic elution with Methanol : Water:,Acetonitrile 30:50:20(v/v/v) was selected with a flow rate of 1.0 ml min^{-1}.The detection wavelength was set at 248nm with a runtime of 7.33mins. The mobile phase was prepared freshly and it was degassed by sonicating for 5 mins before use. The column was equilibrated for at least 30mins with the mobile phase flowing through the system. The column and the HPLC system were kept at ambient temperature.

Preparation of Stock and working standard solutions:

10mg of Ezogabine standard was weighed and dissolved in 10ml of methanol. The resultant solution was filtered through 0.45µm membrane filter paper. From this 1ml of the solution was made up to 10ml to get a concentration of 100µg/ml. From this selected concentrations were prepared by proper dilution.

Preparation of sample solution:

Twenty tablets of Ezogabine (POTIGA- 200mg) were powdered and weighed accurately and a quantity of tablet powder equivalent to 10mg of Ezogabine was dissolved in 10ml of methanol with the

aid of ultra-sonication for 15 min and solution was filtered through 0.45µm membrane filter paper into a 10ml volumetric flask. From the filtrate, appropriate dilution was done in mobile phase to get a solution of 60µg/ml of Ezogabine. From this solution 20µl of the sample was injected into the system to get the chromatogram.

Optimized chromatographic conditions of Ezogabine are given in Table 1 and the chromatogram of the Ezogabine standard is given in figure 2.

Table 1: Optimized chromatographic conditions for the estimation of Ezogabine

Mobile phase	**:** Methanol: Water : Acetonitrile: 30:50:20 (v/v/v)
Pump mode	**:** Isocratic
pH	**:** 5.4 (adjusted with 0.1% OPA)
Diluent	**:** Mobile phase
Column	**:** Zodiac C18 column (250 mm X 4.6 mm I.D.,5µm particle size)
Column Temp	**:** Ambient
Wavelength	**:** 248nm
Injection Volume	**:** 20µL
Flow rate	**:** 1.0mL/min
Run time	**:** 10mins
Typical t_R of Ezogabine :	7.33mins

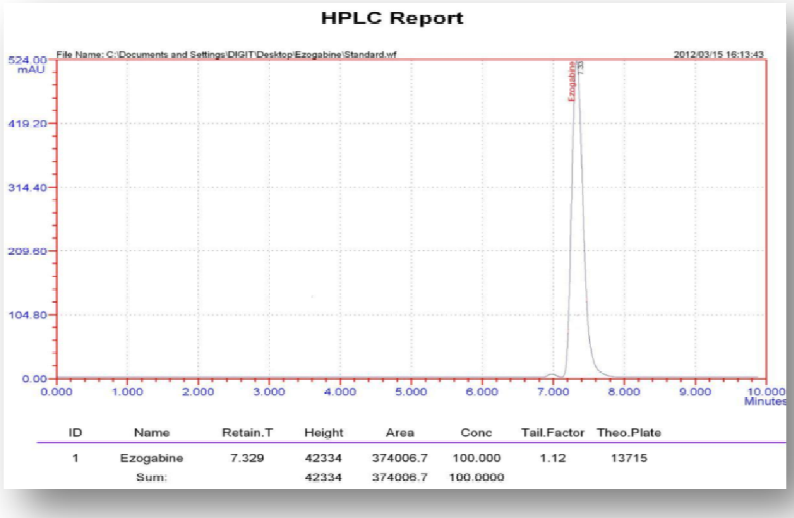

Figure 2 chromatogram of Standard Solution

Method Validation procedure

The objective of the method validation is to demonstrate that the method is suitable for its intended purpose as it is stated in ICH guidelines. The method was validated for linearity, precision, accuracy, specificity, and limit of detection, limit of quantification, robustness and system suitability.

Linearity:.20µL of working standard solutions of 30,40,50,60,70,80 µg/ml of Ezogabine was injected into the chromatographic system. The chromatograms were developed and the peak area was determined for each concentration of the drug solution. Calibration curve of Ezogabine was obtained by plotting the peak area ratio versus the applied concentrations of Ezogabine. The linear correlation coefficient was found to be 0.999.

Table 2: Linearity results of Ezogabine

Levels	Concentration of Ezogabine in µg/ml	Peak area
Level1	30	189631
Level 2	40	247539
Level 3	50	312057
Level 4	60	379260
Level 5	70	445568
Level 6	80	510809
	Slope Intercept Correlation Coefficient	6477 -8793 0.999

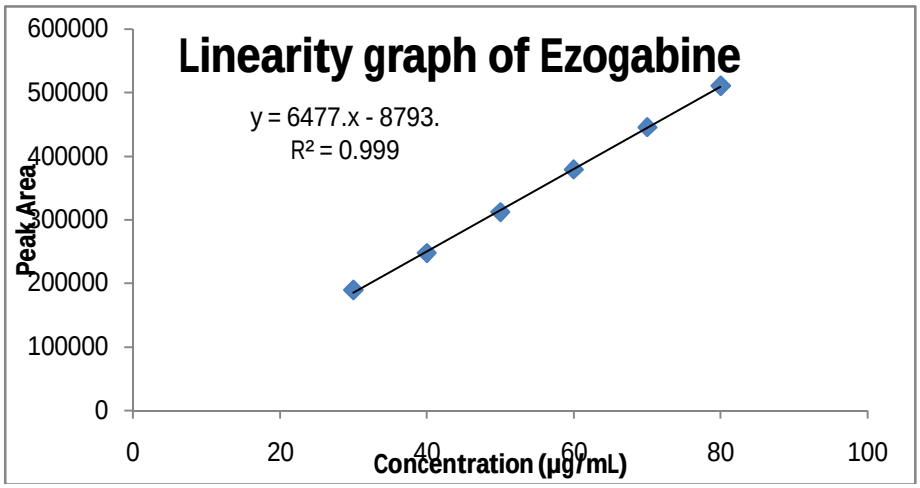

Figure 3: Linearity plot of Ezogabine

Precision : Repeatability of the method was checked by injecting replicate injections of 60 ppm of the solution for six times on the same day as intraday precision and for three consecutive days for inter day precision study of Ezogabine and the %RSD for intraday is found to be 0.1% and for inter day 0.16%.

Table 3: Precision parameters of Ezogabine

INJECTION	INTERDAY	INTRADAY
1	374398	374987
2	375102	374268
3	375026	374398
4	374987	375219
5	374093	375632
6	374721	374145
	%R.S.D =0.1%	%R.S.D =0.16%

Intermediate precision or Ruggedness: Ruggedness of the method was determined by using six replicate injections of standard and sample solutions of same concentrations which were prepared and analysed by different analysts on three different days over a period of one week. Ruggedness is expressed in terms of %RSD. The results are given in table 4.

Table 4:Ruggedness results of Ezogabine

Sample	Conc. (in µg/ml)	Injection No.	Peak Areas	%RSD (Acceptance criteria ≤ 2.0%)
Ezogabine	60	1	374687	0.11
		2	374687	
		3	374011	
		4	375069	
		5	374267	
		6	374086	

Accuracy: The accuracy of the method was determined by calculating recovery of Ezogabine by the method of standard addition. Known amount of Ezogabine was added to a pre quantified sample solution and the amount of Ezogabine was estimated by measuring the peak area ratios and by fitting these values to the straight line equation of calibration curve. The recovery studies were carried out three times over the specified concentration range and amount of Ezogabine was estimated by measuring the peak area ratios by fitting these values to the straight line equation of calibration curve. From the above determination, percentage recovery and standard deviation of percentage recovery were calculated.

Table 5 :Recovery results of Ezogabine

% Recovery	Ezogabine					
	Target Conc., (µg/ml)	Spiked conc, (µg/ml)	Final Conc, (µg/ml)	Conc., Obtained	% of Recovery	RSD
50%	40	10	50	49.24	98.48	0.44
	40	10	50	49.09	98.18	
	40	10	50	49.52	99.04	
100%	40	20	60	59.63	99.38	0.90
	40	20	60	59.26	98.77	
	40	20	60	60.32	100.53	
150%	40	40	80	79.56	99.45	0.98
	40	40	80	80.63	100.78	
	40	40	80	81.09	101.36	

Robustness: To determine the robustness of the method, two or three parameters from the optimized chromatographic conditions were varied.

Table 6 : Robustness results of Ezogabine

Condition	%Assay	%Difference
Unaltered	100	
Wavelength at 246nm	99.92	0.08
Wavelength at 250nm	100.10	0.10
Mobile phase:		
Methanol(35):Water(45): ACN (20) v/v	100.45	0.45
Methanol(25):Water(55): ACN (20) v/v	100.40	0.40
P^H – 5.3	100.78	0.78
P^H – 5.5	100.66	0.66

System Suitability: System suitability was studied under each validation parameters by injecting six replicates of the standard solution. The system suitability parameters are given in Table7.

Table 7: System suitability parameters

Parameter	Tailing factor	Theoretical plates
Specificity study	1.12	13714
Linearity study	1.24	13762
Precision study	1.06	13651

LOD and LOQ

Limit of detection (LOD) and limit of quantification (LOQ) were found to be 0.09ppm and 0.3ppm respectively as per ICH guide-lines.

Stability test

To perform the Stability test the standard solution of 60µg/ml was stored at ambient temperature (±10^0C) for two days. After this these storage solutions and freshly prepared solution were tested with proposed method. It is noticed that assay of these results were did not decreased below 98%. The results of stability test were shown in Table 8.

Table 8: Stability results of Ezogabine

Standard solution			Sample solution		
Time (hours)	**Peak area**	**%variation**	**Time (hours)**	**Peak area**	**%variation**
Initial	374006	---	Initial	368770	---
12	373931	0.03	12	366246	0.61
24	373574	0.12	24	365049	1.01

Assay of formulation of Ezogabine: Ezogabine (POTIGA- 100 mg) ,20 tablets were weighed and the average weight was calculated. Accurately weighed and transferred the sample equivalent to 10mg of Ezogabine in to a 10ml volumetric flask. Diluent is added and sonicated to dissolve it completely and made volume up to the mark with diluents. Mixed well and filtered through 0.45µm membrane filter paper. Further pipetted 1ml of the above stock solution into a 10ml volumetric flask and diluted up to mark with diluents and finally 60µg/ml solution was prepared. Mixed well and filtered through 0.45µm membrane filter paper. An aliquot of this solution was injected into HPLC system. Peak area of Ezogabine was measured for the determination.

Table 9:Assay Result

Formulation	Tablet dosage	Sample concentration	Drug estimated	% of Drug estimated
POTIGA	100mg	60ppm	59.14	98.6

RESULTS AND DISCUSSION

The drug Ezogabine was analysed by C18 column. Different mobile phases were tried but satisfactory separation, well resolved and good symmetrical peaks were obtained with the mobile phase Methanol:Water:Acetonitrile 30:50:20 (v/v/v). The retention time of Ezogabine was found to be 7.33mins. The %RSD values for accuracy and precision studies obtained were less than 2% which revealed that developed method was accurate and precise. The system suitability parameters are given in Table 7. The percentage of recovery of Ezogabine was found to be in the range 98.18%-100.78% indicating that the proposed method is highly accurate. Proposed liquid chromatographic method was applied for the determination of Ezogabine in tablet formulation.The result for Ezogabine was comparable with a corresponding labelled amount . The absence of additional peaks indicates no interference of the excipients used in the tablets.

CONCLUSION

A validated RP-HPLC method has been developed for the determination of Ezogabine tablet dosage form. The proposed method is simple, rapid, accurate, precise and specific. Its chromatographic run time of 10 min allows the analysis of a large number of samples in short period of time. Therefore, it is suitable for the routine analysis of Ezogabine pharmaceutical dosage form.

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