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Development and validation of gradient stability indicating HPLC method for determining Ezogabine and related substances

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ABSTRACT

A high performance liquid chromatographic method is presented for the determination of Ezogabine and its impurities in the presence of its degradation products using a reverse phase C18 column at ambient temperature with mobile consisting of 0.01M di potassium hydrogen phosphate pH 7.6 and acetonitrile. The flow rate was 0.8ml/min. Quantitation was achieved with wavelength at 254nm. The proposed method was validated for specificity, linearity, accuracy, LOD, LOQ, precision and robustness. The method was found to be suitable for the quality control of Ezogabine in bulk drug and dosage form as well as the stability indicating.

INTRODUCTION

Ezogabine is an anticonvulsant used as an adjunctive treatment for partial epilepsies in treatment experienced adult patients[1]. Ezogabine (ethyl (2-amino-4-((4-fluorobenzyl) amino) phenyl) carbamate works primarily as a potassium channel opener, by activating a certain family of voltage gated potassium channels in the brain[2-4].

The literature survey reveals that Ezogabine has been determined using a number of techniques including LC-MS[5-8], potentiometry[9], residual solvents by GC[10], and assay by HPLC[11-15]. The aim of this work was to develop stability indicating methods for determination of Ezogabine and its impurities in the presence of its degradants using HPLC in bulk drag and dosage form.

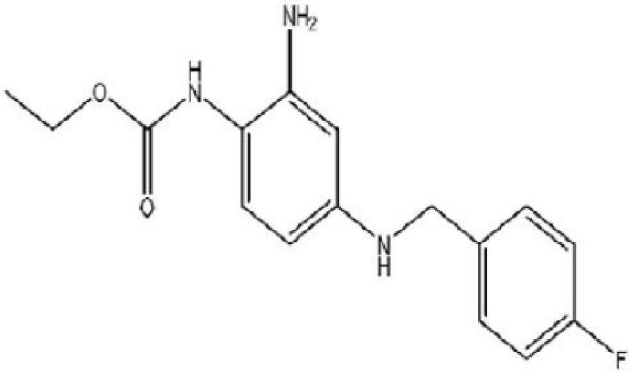
EXPERIMENTAL

Chemicals & reagents

The HPLC grade acetonitrile and analytical grade di potassium hydrogen phosphate and phosphoric acid were purchased from Merck, Mumbai. High purity water was prepared by using a Millipore Milli Q plus water purification system. Pure of all impurities namely IMP-I, IMP-II, IMP-III, and Ezogabine as a gift.

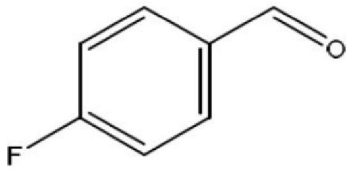
Chromatographic conditions

All analyses were performed using Shimadzu 2010 equipped with UV detector with empower software. Buffer consisted of 0.01M dipotassium hydrogen phos-



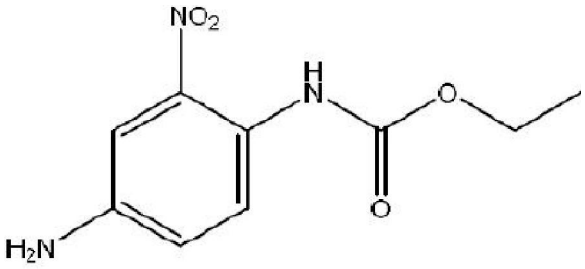
ethyl (2-amino-4-((4-fluorobenzyl)amino)phenyl)carbamate

Figure 1 : Chemical structure of Ezogabine



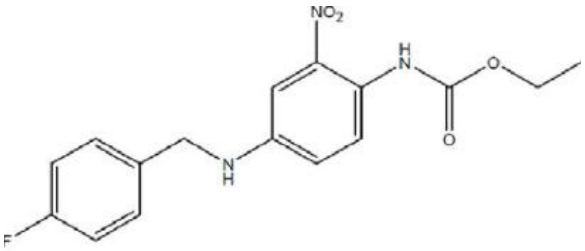
4-fluorobenzaldehyde

Figure 2 : impurity-I



ethyl (4-amino-2-nitrophenyl)carbamate

Figure 3 : impurity-II



ethyl (4-((4-fluorobenzyl)amino)-2-nitrophenyl)carbamate

Figure 4 : Impurity-III

phate in water (1.74g of K2HPO4 in 1000 ml of water) pH adjusted 7.6 with phosphoric acid. AnXBridge C18 (4.6x150) mm, 3.5 µm column and gradient mixture of solution A (Buffer) solution B (Acetonitrile) used as stationary and mobile phase respectively. The gradient program was set as (T/%B) 0/30, 45/70, 50/70, 51/30 and 60/30. Acetonitrile used as diluent. The column oven maintained at 25°c with a 0.8 ml flow rate. An injection volume 10µl was used. The elution compounds were monitored at 254 nm.

Preparation of standardand sample solutions

A stock solution of Ezogabine (mg/ml) is prepared by dissolving the appropriate amount of Ezogabine solid in the diluent. Working solutions of 0.10% and for impurities 0.10% are prepared from the stock solution for the determination of related substances respectively. The drug product equivalent to 100mg of sample is transferred to 100ml flask dissolved and diluent volume with diluent (mg/ml). this solution then filtered through a 0.45µ nylon membrane filter.

RESULTS AND DISCUSSION

Method development optimization

The main objective of the method was to separate

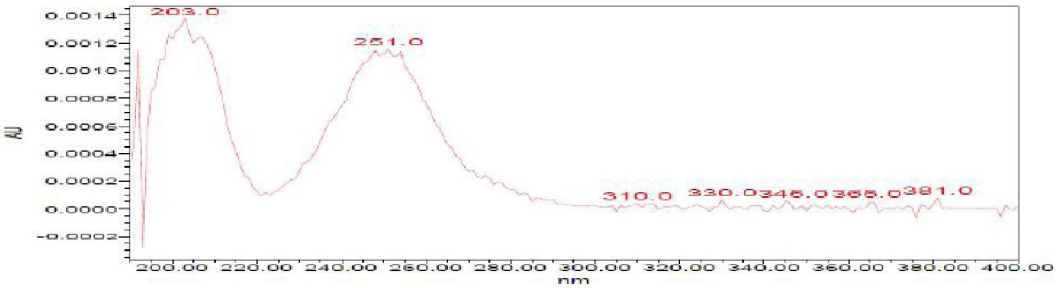


Figure 5 : Wavelength spectrum of impurity-I

Ezogabine from its known impurities and degradants by reverse-phase chromatography. Ezogabine and its related impurities wavelength was selected as 254nm Figure (5-9) at this wavelength impurity are shown maximum absorbs. Mobile phase selected as dipotassium hydrogen phosphate, pH 7.6 with phosphoric acid, in low pH Ezogabine and impurities were co-eluted. Different column such as zorbax XDB-C18, Inertsil ODS columns are used, but Ezogabine peak was not sharp. In XBridge C18 (4.6x150) mm, 3.5 µm column Ezogabine and its impurities are sharp and well resolved. The gradient program was set as (T/%B) 0/30, 45/70, 50/70, 51/30 and 60/30. Acetonitrile used as diluent. The column oven maintained at 25Úc with a 0.8 ml flow rate. An injection volume 10µl was used. After many logical trials, chromatographic condition was established such that which could be suitable for separation of drug-degradation products and drug-three known impurities. Using the optimized conditions, Ezogabine and its known impurities were separated with a resolution of greater than 2. The system suitability, results are given in TABLE 1.

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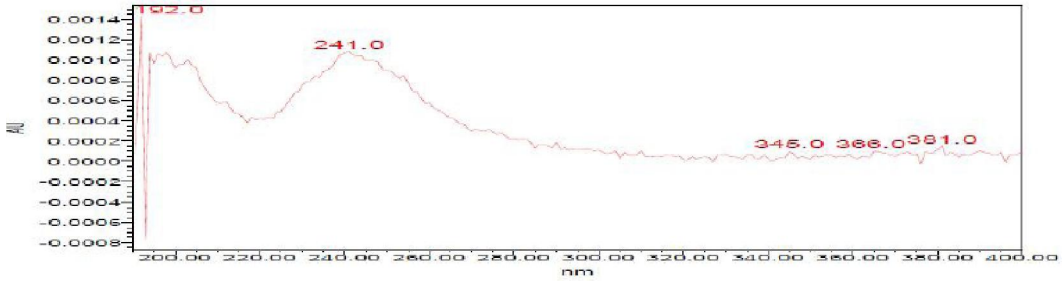

Figure 6 : Wavelength spectrum of impurity-II

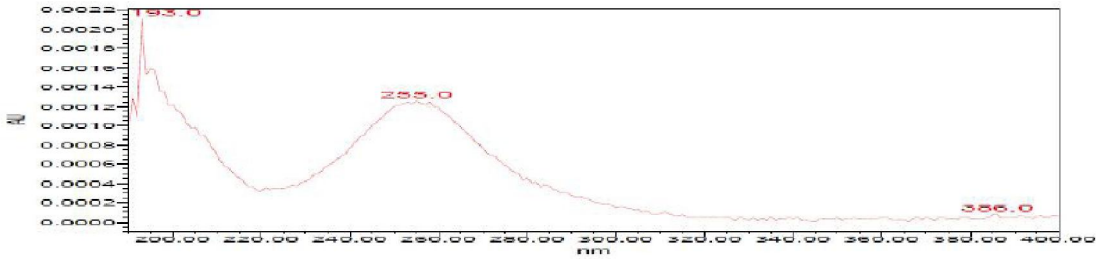

Figure 7 : Wavelength spectrum of impurity- III

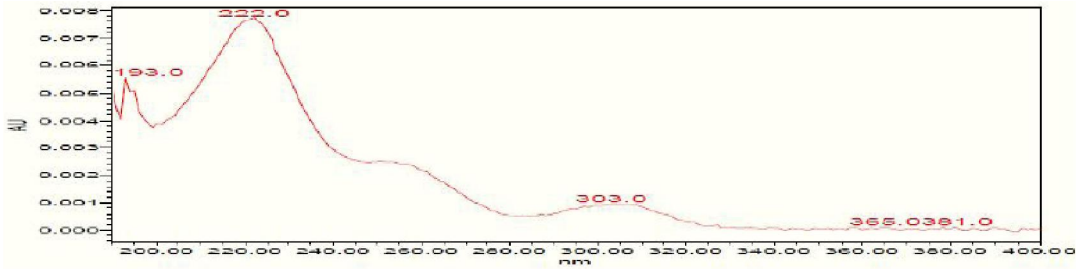

Figure 8 : Wavelength spectrum of Ezogabine

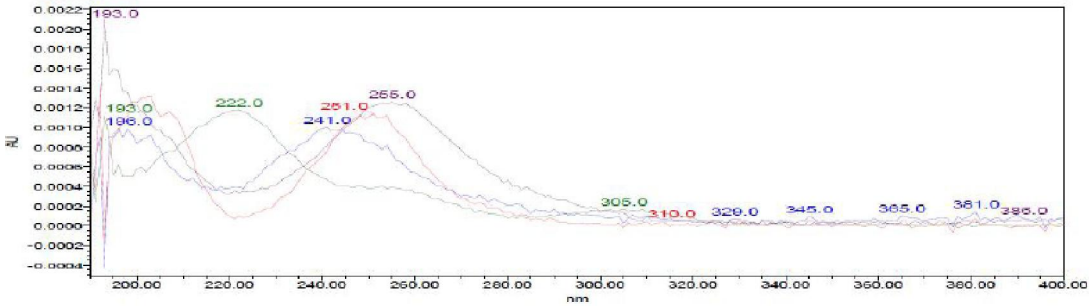

Figure 9 : Compare wavelength spectrum

Analytical method validation

The proposed method was validated as per ICH guidelines[16].

Specificity

It is performed by injecting individual impurities and found well separated with an Ezogabine drug substance known and unknown impurities. To further evaluate the stability indicating the power of the analytical method, Ezogabine was subjected to stress testing as per ICH recommended test conditions. Intentional degradation was attempted with a stress condition of UV light (1.2 Million Lux hours), heat (80ºC, 7days), acid (0.1N HCl, 80ºC, 24hrs), base (0.1N NaOH, 12hrs, 80ºC), oxidation (3% H2O2, 24hrs room temperature). Ezogabine was found to degrade significantly in base hydrolysis and oxidation, whereas the impurities are found well separated and found method is specific.

Precision and intermediate precision

TABLE 1: Validation results

Validation parameter	Imp-I	Imp-II	Imp-III	Ezogabine
System Precision				
%RSD of peak area	0.57	0.35	0.34	0.47
Resolution		2.2	35.7	11.26
Tailing factor	1.02	1.11	1.13	1.11
Column efficiency	7454	8863	58024	17233
Linearity				
Slope	148787	83494	96557	39630
Intercept	0.2	0.4	-2.3	-0.7
r2	0.9995	0.9999	0.9985	0.9996
Quantitation limit (%)	0.005	0.008	0.010	0.015
Detection limit (%)	0.002	0.002	0.003	0.005
Precision at QL	2.1	2.3	1.7	2.0
Accuracy mean%recovery at				
QL	98.8	97.2	98.7	NA
50%	98.6	100.0	98.5	NA
100%	98.6	99.5	99.4	NA
150%	100.0	101.0	100.4	NA
Method precision	1.1	0.6	0.4	
Intermediate precision				
%RSD	0.7	0.6	0.7	NA

The precision of the method repeatability was verified injecting six individual preparations of Ezogabine (mg/ml) spiked with 0.10% of its three impurities. The intermediate precision of the method was also evaluated using six independent sample preparations spiked to 0.10% of its impurities at 100% of the target concentrations as defined by the method. The precision %RSD for the content of all three impurities were less than 2%. The results obtained in the intermediate precision study for the %RSD of the three impurities were less than 3.0%, confirming the high precision of the method. The results are shown in TABLE 1.

Sensitivity

The LOD was calculated for each compound as a signal-to-noise ratio (S/N) of approximately 3:1. It was determined by serial dilution of standard solution. While the LOQ should be estimated as an S/N of approximately 10:1. The limit of detection of imp-I, imp-II, and imp-III were 0.002, 0.002, and 0.003, (of analyte concentration mg/ml). Under the same conditions, the LOQ were 0.005, 0.008, and0.010. The precision of all impurities LOQ was less than 2%. The results are shown in TABLE 1.

Linearity and range

Under the optimum conditions linearity was studied using six concentration ranges from LOQ to 150% for Imp-I, Imp-II, Imp-III and Ezogabine. The calibration curves were developed by plotting peak area versus concentration (n=6). The Linear calibration plot for the method was obtained over the calibration ranges tested from LOQ to 150% for imp-I, imp-II, imp-III and Ezogabine. The correlation coefficient obtained was greater than 0.999 for all three impurities and Ezogabine. The results are shown in TABLE 1.

Accuracy

The accuracy studies were performed to verify the closeness of agreement between the expected value and the value found. In the case of chemical purity method model solutions were prepared at 3concentration levels of the related substance. Close to the LOQ, 0.05%, 0.1% and 0.15% of the specification level. The recovery is further calculated by using formula and the results were found to be up to the mark and tabled in TABLE 1. Recovery of the three impurities in Ezogabine ranged

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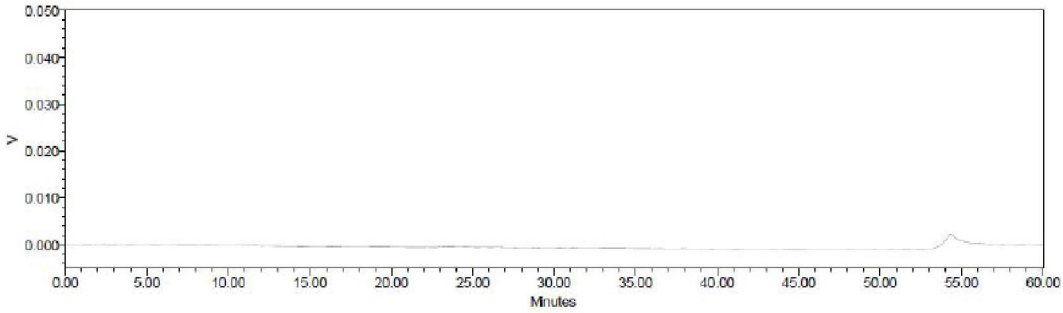
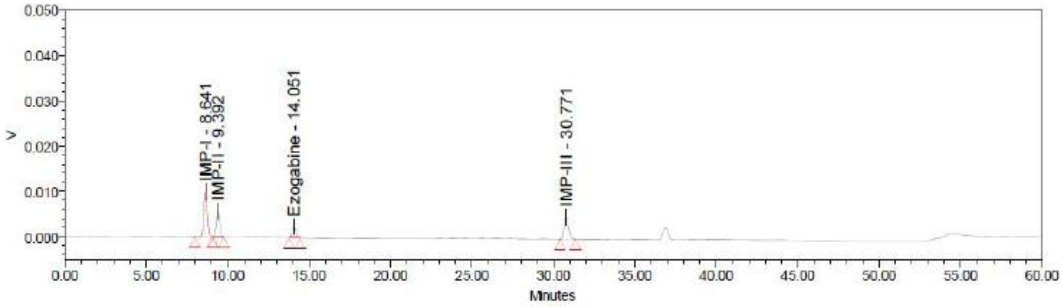
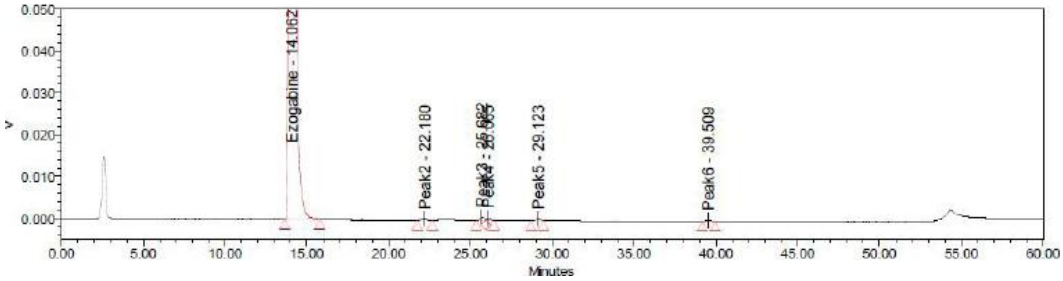


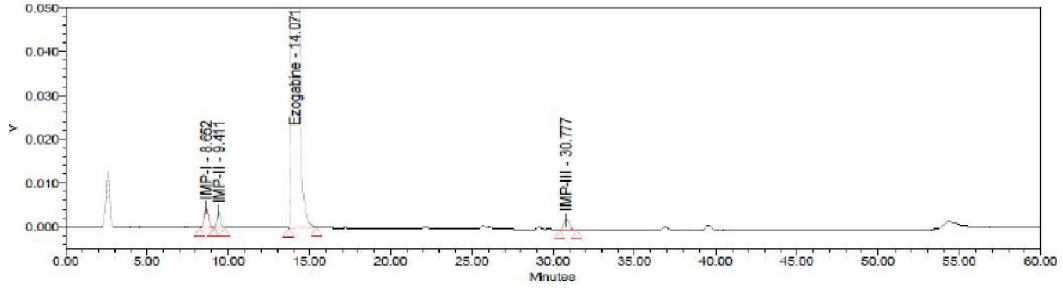
(a)



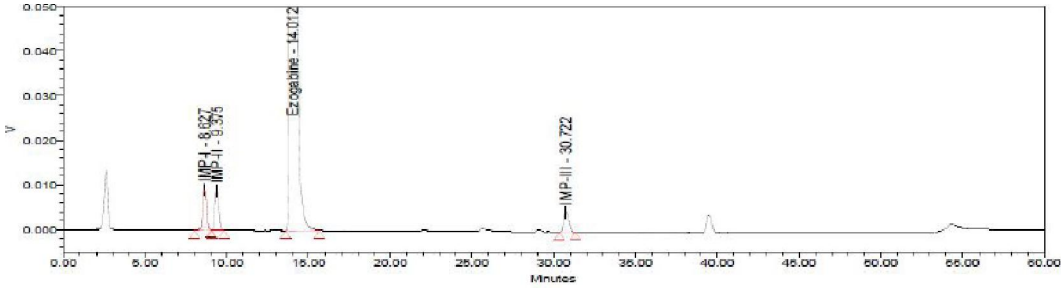
(b)



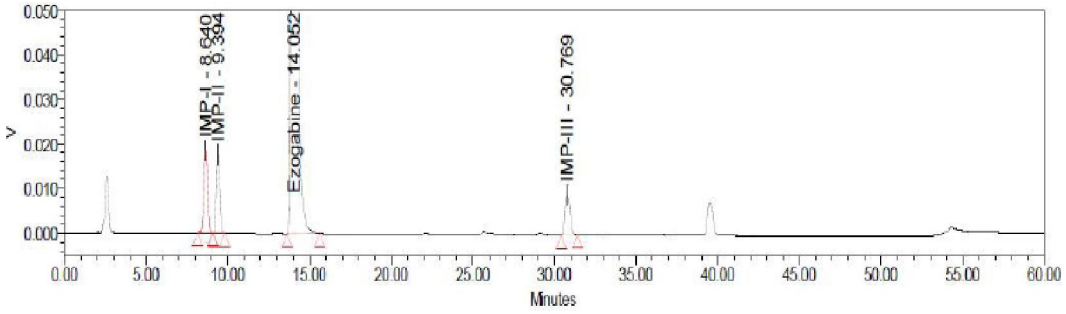
(c)



(d)



(e)



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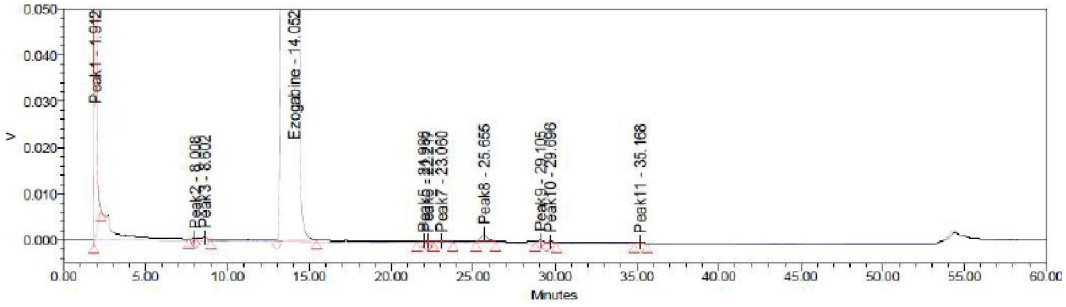
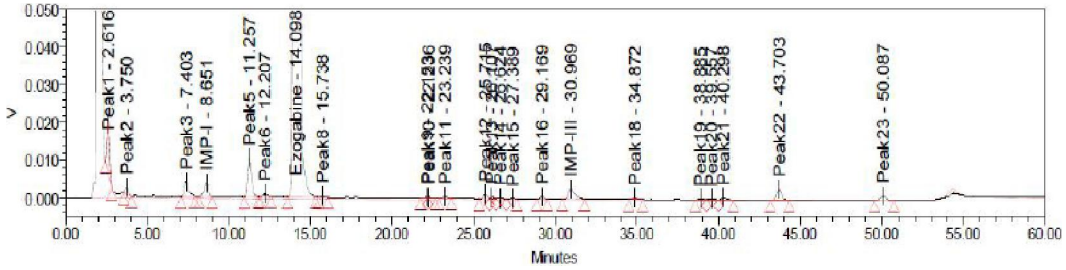
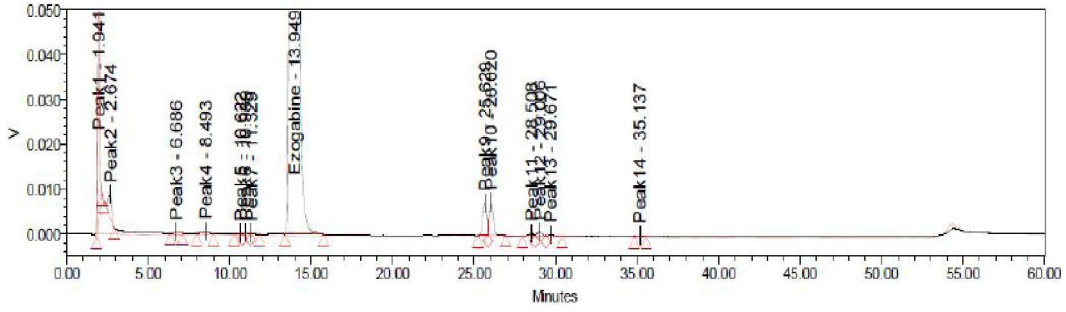


(g)



(h)



(i)

Figure 10 : Typical chromatograms of (a) Blank; (b) standard; (c) sample; (d) 50% spiked; (e) 100% spiked; (f) 150% spiked; (g) acid; (h) base; (i) oxidation

from 97.2 to 101.0. The results are shown in TABLE 1, and it is observed that the method is accurate within the determined range.

Robustness

Deliberate variations in method parameters were done to the robustness of the related compound method evaluate method reliability. The flow rate of the mobile phase was 0.8ml/min, to study the effect of flow rate on the resolution; it was changed by 0.1 units from 0.7 to 0.9 ml/min. The pH of the mobile phase was 7.6; to study the pH on the resolution was studied 7.5 and 7.7. Close observation of analysis results for deliberately

changed chromatographic conditions (flow rate and pH) revealed that the resolution between closely eluting impurities, namely Imp-I and Imp-II has been always greater than 2.0, illustrating the robustness of the method.

Solution stability

Solution stability of Ezogabine and its Impurities in the related substance, method was carried out by spiked sample solutions in tightly capped volumetric flasks at room temperature for 48hrs. Content of impurities Imp-I, Imp-II, Imp-III and Ezogabine were determined for every 24hrs interval up to the study period. No significant changes were observed in the content of imp-I, imp-II and imp-III, during the solution stability and mobile phase stability experiments. Date confirms that samples solutions and mobile phase stability used during related substance, determination were stable up to the study period of 48hrs.

CONCLUSION

A simple, specific, linear, precise, and accurate RP-HPLC method has been developed and validated for determination of Ezogabine and its impurities in bulk drug and dosage form. The method was successfully validated in accordance with ICH guidelines. The fine validation results showed that the proposed method could also considered as a reliable, convenient and sensitive quality control supplement for the routine determinations of Ezogabine in bulk drug and dosage form.

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