



Simple novel UV-spectroscopic method for estimation of Ezetimibe in tablet dosage form

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ABSTRACT

A simple, accurate and precise UV-spectrophotometric method was developed for determination of Ezetimibe in bulk and tablet dosage form. This method based on determination of ezetimibe in ethanol: acetic acid (90:10) at 252 nm. Regression analysis of Beer's plots showed good correlation in concentration range of 2- 20 µg/ml for Ezetimibe. The % recovery was between 100.9 to 102.32% indicating high degree of accuracy of the proposed methods. The results of the analysis were validated statistically and recovery studies were carried out as per ICH guidelines.

Keywords: Ezetimibe, UV-Spectroscopic method, recovery studies

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INTRODUCTION

Ezetimibe (EZ), 1- (4-Fluorophenyl) – 3 (R) - [3-(4-fluorophenyl) - 3 (S) hydroxyl propyl]-4 (S) –(4-hydroxy phenyl) – 2 azetidiones is a therapeutically beneficial drug that works by inhibiting the protein transporters on small intestinal brush border, which brings about this active transport of cholesterol. In addition, it also inhibits phytosterol absorption³. EZ has no inhibitory effect on absorption of lipid soluble vitamin¹⁻⁵. A literature survey revealed that a few UV spectroscopic methods⁶, HPLC and LC-MS⁷ methods have been reported for the estimation of EZE in pharmaceutical formulations and in plasma. Also, HPLC⁸, methods were reported for the estimation of EZE in tablet dosage form. The review of literature revealed that no method is reported for the estimation of EZE in fixed dose products by UV spectrophotometry. The present research describes a simple, rapid, accurate and reproducible, economic method for the estimation of EZE in tablet formulation

MATERIALS AND METHODS

Equipment and reagents

A Shimadzu model 1700 double beam UV-Visible Spectrophotometer with two matched cuvette cells of one cm light path were used for the measurement of absorbance. The Ezetimibe bulk drug was kindly gifted by Lupine Research Pvt. Limited, Pune. The pharmaceutical dosage form was procured from market. Ethanol, acetic acid Whatmann filter paper of AR grade was used in study.

Preparation of standard stock solution

Accurately weighed 10 mg of Ezetimibe was transferred into 100 ml volumetric flask volume was made up to 100 ml with Ethanol & Glacial acetic acid in the ratio of 90: 10 to get a concentration of 100 µg/ml. The prepared solution is sonicated for 10 minutes and filtered through the Whatman filter paper no.41.

Concentration of calibration curve

Aliquots of standard solution were pipette out and suitably diluted with mixture of ethanol: acetic acid in ratio 90:10 to get final concentration of 2-20 µg/ml. The solution was scanned in spectrum mode of 400 to 200 nm wavelength range and sharp peak obtained at 252 nm shown in Figure.1 Calibration curve was plotted absorbance against concentration and regression equation was computed. The results tabulated in the table 1.

Table 1: Optical parameters of Ezetimibe

Parameter	Value
Working λ max	252 nm
Beer's Law Limit	2-18 μ g/ml
Intercept*	0.030
Slope*	0.05
Correlation coefficient	0.998
Regression Equation	$Y=0.05x+0.030$

Assay of Ezetimibe

Twenty tablets of brand EZEDOC containing 10 mg of Ezetimibe were weighed, average weight determined and finely powdered with the help of mortar and pestle. Appropriate quantity of powder from each capsule equivalent to 10 mg of Ezetimibe was accurately weighed transferred to a 100 ml volumetric flask and volume was made up to 100 ml with Ethanol & Glacial acetic acid in the ratio of 90:10. Shaken vigorously for 15 min. and filtered through the Whatman filter paper no.41. Necessary dilutions of filtrate were made with Ethanol and to get Glacial acetic acid concentration 20 μ g/ml of Ezedoc. Absorbance of this solution was measured at 252 nm.

RESULT AND DISCUSSION

The development of a simple, rapid, sensitive, and accurate analytical method for the routine quantitative determination of samples will reduce unnecessary tedious sample preparations, the cost of materials and labor. Ezetimibe is a UV-absorbing molecule which absorb at a particular wavelength and this fact was successfully employed for their quantitative determinations using the UV spectrophotometric method. The absorption spectrum of Ezetimibe in Ethanol & Glacial acetic was shown in Figure 1. Calibration curve data was constructed in the range of the concentrations of 2-20 μ g/ml. The regression equation was found to be $y = 0.054x + 0.030$.

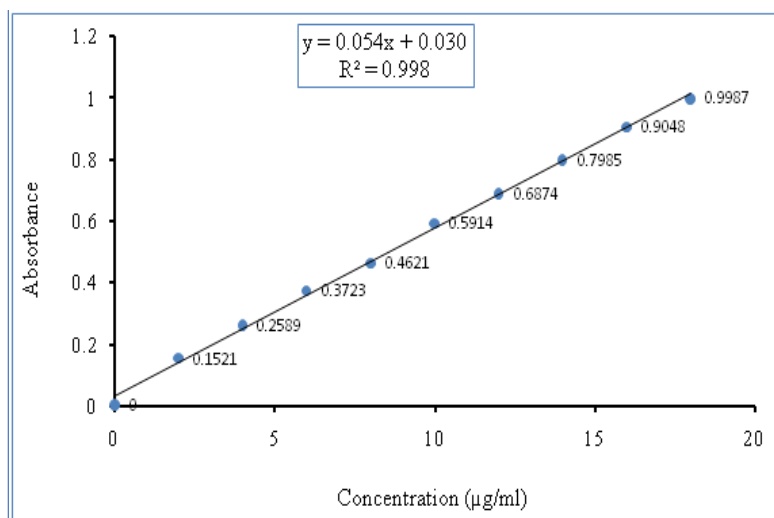


Figure 1: Calibration curve of Ezetimibe at 252nm

The correlation coefficient (r^2) of the standard curve was found to be 0.998. The stock solutions and working standards were made in Ethanol & Glacial acetic acid in the ratio of 90:10. The λ_{max} of the drug for analysis was determined by taking scans of the drug sample solutions in the entire UV region. Performing replicate analyses of the standard solutions was used to assess the accuracy, precision, and reproducibility of the proposed method. The selected concentration within the calibration range was prepared in Ethanol & Glacial acetic acid in the ratio of 90:10 and analyzed with the relevant calibration curve to determine the intra and inter day variability. The proposed method can be successfully applied for assay in tablet dosage forms without any interference. The assay showed that the drug content of this product to be in accordance with the labeled claim (Table 2). The recovery of the analyte of interest from a given matrix can be used as a measure of the accuracy of the method (Table 3).

Table 2: Determination of Ezetimibe

Sr. No	Tablet Name	Drug Name	Label Claim in mg	%Label Found*	Claim Amount Found in mg
1	Ezedoc	Ezetimibe	10mg	98.62	9.86

Table 3: % Recovery Result

Recovery Level	Concentration $\mu\text{g/ml}^*$	% Recovery*	S.D.	% RSD
80%	10	102.32	0.094	0.1675
100%	12	102.0	0.122	0.2435
120%	14	100.9	0.049	0.5432

S.D=Standard deviation, RSD=Relative Standard deviation.

In order to check the accuracy and precision of the developed method and to prove the absence of interference by excipients, recovery studies were carried out after the addition of known amounts of the pure drug to various pre-analyzed formulations of all drugs. These results reveal that the developed method have an adequate precision and accuracy, and consequently, can be applied to the determination of Ezetimibe tablet in pharmaceuticals without any interference from the excipients.

Validation of method parameters

Linearity

Developed method validated as per ICH guidelines⁹. The plot of absorbance against concentration was Plotted and plot is linear over the concentration range of 2-20 $\mu\text{g/ml}$ with (r^2) 0.998 shown in Figure 2.

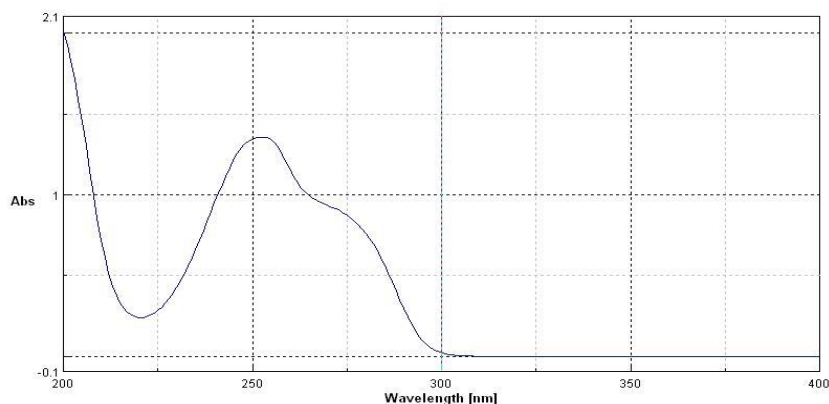


Figure.2: Spectrum of pure Ezetimibe

Precision

Interday and intraday precision was determined by repeating assay for three times on same day and on three different day. The relative standard deviation for replicates of sample solution was less than 2.0% which meets acceptance criteria for established method. The obtained results are presented in Table 4.

Table 4: Precision Result

Intraday			
Concentration($\mu\text{g/ml}^*$)	Absorbance	S.D.	%RSD
6	0.108	0.001528	0.0144
	0.106		
	0.105		
12	0.171	0.00264	0.0152
	0.176		
18	0.175	0.002646	0.01
	0.266		
	0.263		
	0.261		
Interday			
Concentration($\mu\text{g/ml}^*$)	Absorbance	S.D.	%RSD
6	0.104	0.0015	0.0144
	0.106		
	0.109		
12	0.173	0.0070	0.0409
	0.170		
18	0.172	0.0011	0.0044
	0.268		
	0.266		
	0.263		

S.D=Standard deviation, RSD=Relative Standard deviation

Accuracy

To check accuracy of proposed method recovery studies were carried out at 80%, 100%, 120%

of test concentration as per ICH guidelines. Excellent recoveries were obtained at each level that is 102.32, 102.00, 100.9 respectively. The validation results are shown in Table 2 and Table 3.

Limit of detection (LOD)

The LOD was determined using the formula:

$$\text{LOD} = 3.3\sigma/S$$

Where, LOD is Limit of Detection,

σ is the standard deviation of the response of blank,

S is the slope of calibration curve.

The LOD for Ezetimibe was found to be 0.10 $\mu\text{g/ml}$

Limit of Quantitation(LOQ)

The limit of quantitation was determined using the formula:

$$\text{LOQ} = 10\sigma/S$$

Where, LOQ is Limit of Quantitation

σ is the standard deviation of the response of blank,

S is the slope of calibration curve

The LOQ for Ezetimibe was found to be 0.30 $\mu\text{g/ml}$.

CONCLUSION

The simple, novel UV-spectroscopic method was developed for Ezetimibe in bulk and tablet dosage form and result indicates that, the method employed here is very simple, accurate, economic and rapid for routine analyses of the drug Ezetimibe.

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