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DEVELOPMENT AND VALIDATION OF UV SPECTROSCOPIC METHOD FOR THE ESTIMATION OF EZETIMIBE IN BULK AND TABLETS

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ABSTRACT

Development and implementation of a simple, precise, and accurate area under curve spectroscopic method for the estimation of Ezetimibe in bulk and pharmaceutical dosage form. The drug indicates that the maximum absorption (λ max) at 231nm in Acetonitrile solution and AUC in absorption spectrum is measured in the wavelength range from 226 to 236nm at a concentration range of 9-21µg/ml according to Beer's law. Linearity analysis showed an R2 of 0.9997. The % recovery was found to be 99-99.36%. The LOD and LOQ were found to be 0.028 and 0.086µg/ml. The %RSD was found less than 2. The method has been validated according to linearity, precision, accuracy, robustness, ruggedness, LOD and LOQ according to ICH guidelines.

KEYWORDS

Ezetimibe, Area under curve spectroscopy, Validation and Pharmaceutical formulations.

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INTRODUCTION

The area under a curve between two points is found by doing a definite integral between the two points. To find the area under the curve y = f(x) between x = a and x = b, integrate y = f(x) between the limits of a and b. This area can be calculated using integration with given limits.

Ezetimibe is a medication used to treat high blood cholesterol and certain other lipid abnormalities. Generally, it is used together with dietary changes and a statin. Alone, it is less preferred than a statin and it is also available in the fixed combo of Ezetimibe and simvastatin, Atorvastatin and bempedioc acid. It is used in the management and treatment of hypercholesterolemia. Ezetimibe

belongs to selective cholesterol-absorption inhibitors. Ezetimibe was discovered and approved by Harry Davis from USFDA and is currently owned in India by Anant Pharmaceuticals Pvt. Ltd. Ezetimibe was commercially manufactured by Healing Pharma under the brand name of Zetiheal in India.

Clinical Studies

A double-blind, randomized trial involving 18,144 patients who had been hospitalized for acute coronary syndrome within the preceding 10 days and had LDL (low-density lipids) cholesterol levels of 50 to 100 mg per deciliter (1.3 to 2.6 mmol per liter). Composite of cardiovascular death, nonfatal myocardial infarction, unstable angina requiring rehospitalization, coronary revascularization (≥30 days after randomization), or nonfatal stroke. The median follow-up was 6 years. The Ezetimibe and rosuvastatin of 10mg and +40mg in combination.

A literature survey revealed that there were few analytical methods have been reported for the determination of Ezetimibe in pure drug and pharmaceutical dosage forms by using UV spectrophotometric[1-5], HPLC [6-18] and HPTLC[19] so far.

The present work aims to develop and validate a novel, rapid, simple, precise and specific Area under the curve (AUC) Spectrophotometric method for the estimation of Ezetimibe in bulk and tablet dosage form.

MATERIALS AND METHODS

Instrument

UV-Visible double beam spectrophotometer, SHIMADZU (model UV-1800) with UV probe software. All weights were taken in analytical balance.

Chemicals

Ezetimibe pure drug was obtained as a gift sample from Recipharm pharma services Pvt, Ltd. Banglore, India and its pharmaceutical dosage form Ezetimibe of 10 tablets labeled claim 10mg from a local pharmacy manufactured by Ananth Pharmaceuticals Ltd.

Solvent

Acetonitrile was used as a solvent.

Selection of analytical wavelength

Appropriate dilutions of Ezetimibe were prepared from the standard stock solution and using spectrophotometer solution was scanned in the wavelength range 200-400nm. The area under Curve [AUC] in absorption spectra was measured between the wavelength range 226 to 236nm as the detection wavelength (Figure No.3).

Preparation of standard stock solution

100mg of Ezetimibe was weighed accurately and transferred into a 100ml volumetric flask and diluted in Acetonitrile up to mark. From this, 10ml of the solution was further diluted into 100µg/ml and pipette 0.9, 1.2, 1.5, 1.8 and 2.1ml into 10ml individual volumetric flasks and diluted in Acetonitrile up to mark, this gives 9,12,15,18 and 21µg/ml concentration.

Preparation of sample solution

20 tablets of Ezetimibe marketed formulations were weighed and powdered. A required quantity of tablet powder equivalent to 100mg of Ezetimibe was transferred into a 100ml of volumetric flask then it was diluted with Acetonitrile and made up to the mark.

METHOD AND VALIDATION

The method was validated according to ICH guidelines[20-22].

RESULTS AND DISCUSSION

Method

Area under curve spectroscopy.

Linearity

The linearity of an analytical method is its capacity to show test results that are directly proportional to the concentration of the analyte in the sample within the range. The linearity was established in the range of 9-21µg/ml and the Area under Curve [AUC] in absorption spectra was measured between the wavelengths of 226 to 236nm as absorbance values are shown in Table No.1 (Figure No.4). The calibration curve was prepared by plotting a graph against the concentration and absorbance and therefore the graph is shown in (Figure No.5). Statistical parameters like slope, intercept,

regression equation, correlation coefficient, and Sandell's sensitivity were determined. (Table No.2).

Precision

The precision of an analytical method expresses the closeness of a series of individual analyte measurements obtained from multiple sampling of the equivalent sample. Precision was determined by the intra-day and inter-day study. Intra-day precision was determined by analyzing the same concentration six times on the same day. Inter-day precision was determined by analyzing the same concentration daily for six days. (Table No.3).

Accuracy

The accuracy of an analytical method says the closeness of test results obtained by that method to the true value. To assess the accuracy of the developed method, recovery studies were carried out at three different levels 50%, 100%, and 150%. In which the formulation concentration is kept constant and varied pure drug concentration. (Table No.4).

Ruggedness

Ruggedness is defined as the reproducibility of results when the method is performed under variation in conditions. This includes different analysts, laboratories, instruments, temperatures, etc. Ruggedness was determined by different analysts, and the value of %RSD was found to be less than 2. (Table No.5).

LOD and LOQ

The limit of detection in an individual analytical method is the smallest amount of analyte in a sample that can be reliably detected by the analytical method. The limit of quantitation is an individual analytical procedure that is the smallest amount of analyte in a sample that can be quantitatively determined. LOD and LOQ were calculated using a formula.

LOD = 3.3(SD)/S and LOQ = 3(LOD)

LOD and LOQ values of Ezetimibe were found to be 0.0130 and 0.039µg/ml.

Table No.1: Calibration curve at 226-236nm by AUC method

S.No	Concentration in µg/ml	Absorbance ±Standard deviation*
1	0	0
2	9	0.379±0.001528
3	12	0.466±0.002267
4	15	0.608±0.002409
5	18	0.746±0.002
6	21	0.844±0.001675

*Average of six determinations

Table No.2: Regression parameter for Ezetimibe at 226-236nm by AUC method

S.No	Regression parameter	Results
1	Range (µg/ml)	9-21
2	Λmax (nm)	226-236
3	Regression Equation	Y= 0.040x+0.001
4	Slope (b)	0.040
5	Intercept (a)	0.001
6	Correlation Coefficient (r^2)	0.9997
7	Sandell'sequation	0.024
8	Limit of detection (µg/ml)	0.028
9	Limit of quantitation (µg/ml)	0.086

Table No.3: Precision results for Ezetimibe at 270-280nm by AUC method

S.No	Concentration (µg/ml)	Intra-day Absorbance ±Standard deviation*	%RSD**	Inter-day Absorbance ±Standard deviation*	%RSD**
1	2	0.379±0.001528	0.403	0.379±0.005398	1.42
2	4	0.466±0.002267	0.486	0.483±0.006708	1.38
3	6	0.608±0.002409	0.396	0.618±0.001979	0.320
4	8	0.746±0.002	0.268	0.762±0.003197	0.419
5	10	0.884±0.001675	0.198	0.860±0.005878	0.683

*Average of six determinations, **% relative standard deviation.

Table No.4: Accuracy results for Ezetimibe at 226-236 by AUC method

S.No	Spiked Levels	Amount of Sample (µg/ml)	Amount of Standard (µg/ml)	Amount Recovered	% Recovery ±Standard deviation*	%RSD**
1	50	15	7.5	22.36	99.36±0.146	0.146
2	100	15	15	29.75	99±0.346	0.349
3	150	15	22.5	37.27	99.35±0.237	0.238

*Average of six determinations, **% relative standard deviation.

Table No.5: Ruggedness results for Ezetimibe at 226-236 by AUC method

S.No	Analysts	Analyst 1	Analyst 2
1	Mean absorbance	0.608	0.618
2	±Standard deviation*	0.002409	0.001979
3	%RSD	0.396	0.320

*Average of six determinations, **% relative standard deviation.

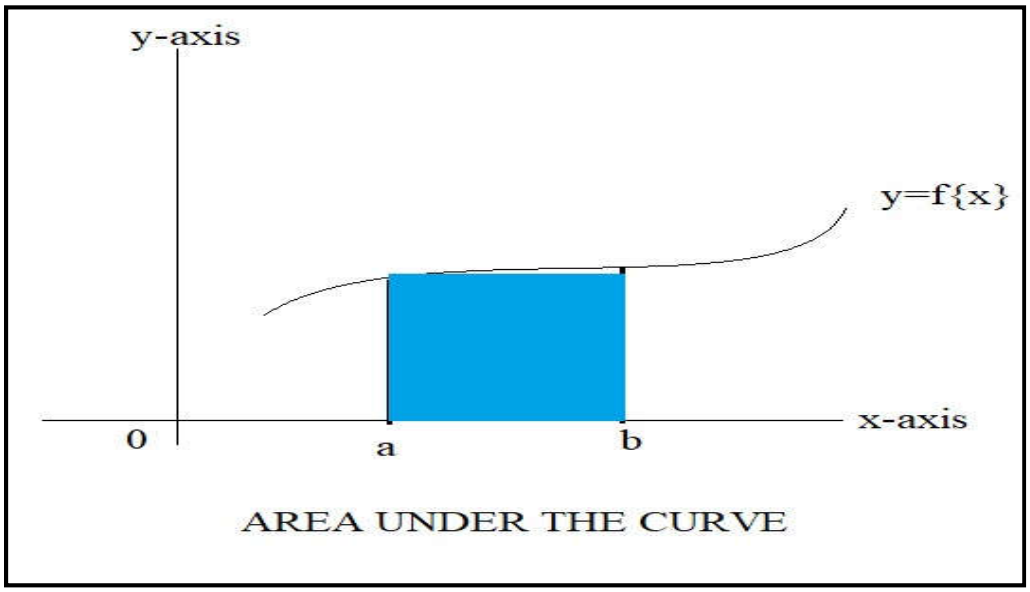

Figure No.1: Area under curve (AUC)

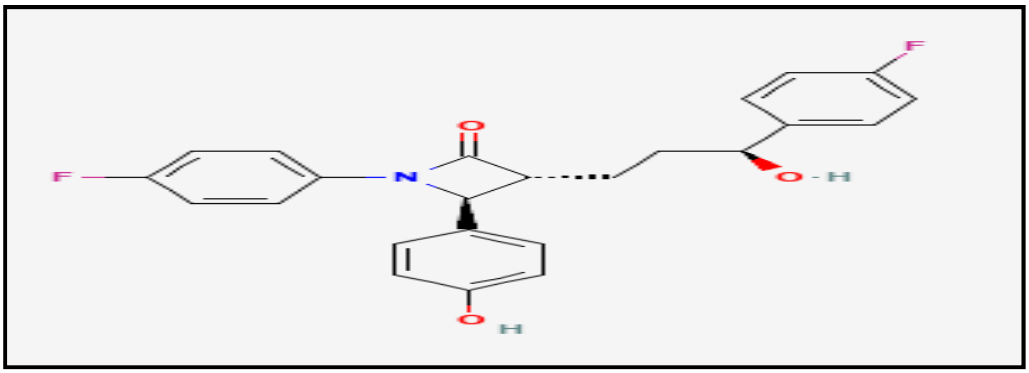

Figure No.2: Chemical structure of Remogliflozin etabonate

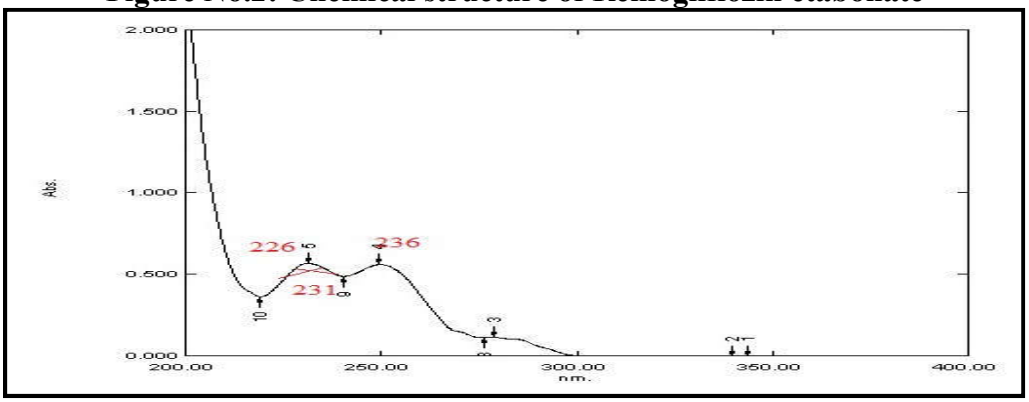

Figure No.3: AUC spectrum of Ezetimibe at 226-236nm

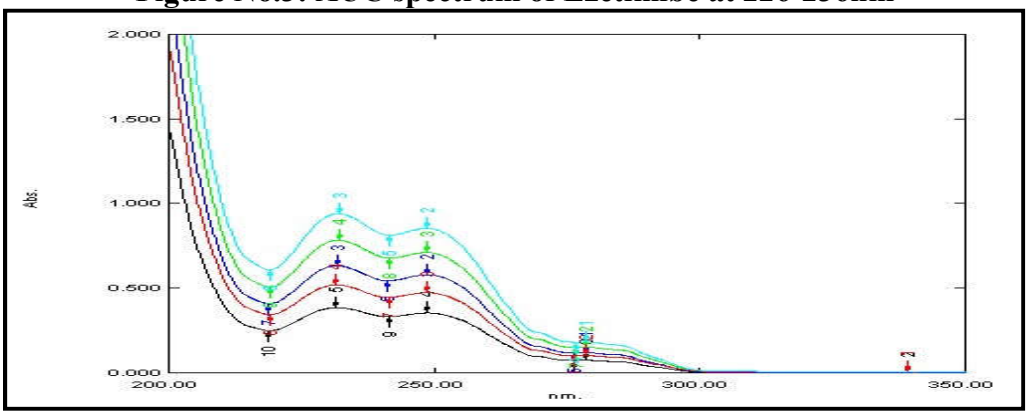

Figure No.4: AUC overlain spectra of Ezetimibe showing absorbance at 226-236nm

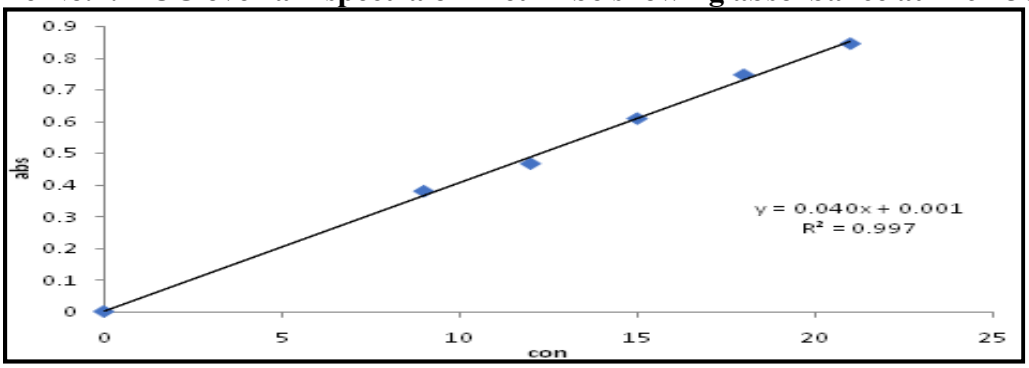

Figure No.5: Calibration curve of Ezetimibe at 226-236nm by AUC method

CONCLUSION

As per ICH guidelines, the present analytical was carried and meet the acceptance criteria. It was concluded that the developed analytical method was simple, specific, accurate, economical and sensitive and can be used for routine analysis of Ezetimibe in bulk drug and in pharmaceutical dosage forms.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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