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Analytical Method Development and Validation of Ezetimibe by Using UV-Spectrophotometric Method

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ABSTRACT

A Novel, specific, accurate, and precise Zero order derivative spectroscopy method was developed and validated for the estimation of Ezetimibe in Bulk and pharmaceutical dosage forms. The stock solution was prepared by weighing 100 mg of standard Ezetimibe in a 100 ml volumetric flask with Ethanol. The stock solution was made to produce 1000 µg/ml with Ethanol. Further dilutions were prepared as per the procedure. The drug solution showed the maximum absorbance at 234 nm. The linearity was found in the concentration range of 2-12 µg/ml. The correlation coefficient was found to be 0.9888. The regression equation was found to be Y=0.053x+0.0238. The method was validated for linearity, accuracy, precision, the limit of detection, limit of quantitation, and ruggedness. The limit of detection and limit of quantitation for the estimation of Ezetimibe was found to be 0.666µg/ml and 6.66µg/ml respectively. Recovery of Ezetimibe was found to be in the range of 100.63-101.73 %. The proposed method was successfully applied for the quantitative determination of Ezetimibe in Bulk and pharmaceutical dosage forms.

INTRODUCTION: [1,2]

Ezetimibe is an anti-hyperlipidemic agent used to lower cholesterol levels1-3 and is official in USP. It is chemically (3R, 4S)-1-(4-Fluoro phenyl)-3-[(3S)-3-(4-floro phenyl]-3- hydroxyl propyl]-4-(4-hydroxy phenyl)-2-azetidinone4. It acts by binding to a critical mediator of cholesterol absorption, the Niemann-Pick C1-Like 1 (NPC1L1) protein on the gastrointestinal tract epithelial cells as well as in hepatocytes. Ezetimibe is in a class of lipid-lowering compounds that selectively inhibits the intestinal absorption of cholesterol and related phytosterols. Ezetimibe, administered alone is indicated as adjunctive therapy to diet for the reduction of elevated total-C, LDL-C, and Apo B in patients with primary hypercholesterolemia. It is also used in combination therapy with HMG-CoA reductase inhibitors. It causes a reduction of hepatic cholesterol stores and an increase in the clearance of cholesterol from the blood. This distinct mechanism is complementary to that of HMG-CoA reductase inhibitors. Various spectrophotometric5-7, HPLC8-10, and LC-MS11-14 methods have been reported for the determination of Ezetimibe in pure and pharmaceutical formulations.

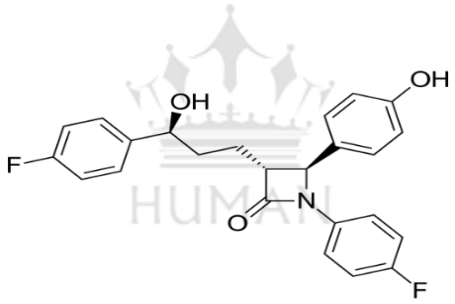

Figure.1: Chemical structure of Ezetimibe

It has a molecular formula of $C_{24}H_{21}FNO_3$ and a molecular weight of 409.4g/mol. It has the structural formula (Fig.1).

Literature Survey revealed that the drug has been estimated by UV spectrophotometric [(3)] and Simultaneous RP- HPLC [(4-9)] methods have been reported so far.

Present work aimed to develop and validate a novel, rapid, simple, precise, and specific Zero order derivative UV-Spectrophotometric method for the estimation of Ezetimibe in its bulk and pharmaceutical dosage form.

MATERIALS AND METHOD:

Instrument:

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

Chemicals:

Ezetimibe was given as a gift sample by Recipharm pharma Services Pvt Ltd, Bangalore, India. Tablets of Ezetimibe were procured from the local market.

Solvent**:** Ethanol

Selection of analytical wavelength:

Appropriate dilutions were prepared for the drug from the standard stock solution and the solution was scanned in the wavelength range of 200-400 nm. The absorption spectra thus obtained were derivatized from the Zero order method. Shows maximum absorbance at 234 nm were shown in Fig.2 and Fig .3.

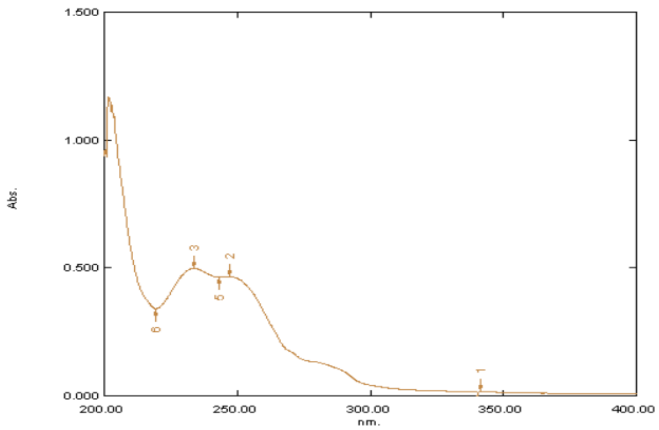

Fig.2: Zero order spectra of Ezetimibe showing absorbance at 234 nm.

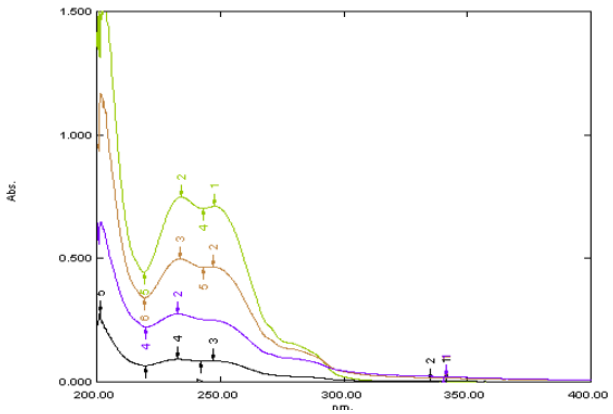

Fig. 3: Zero order overlain spectra of Ezetimibe at 234 nm.

Preparation of Standard solution:

Accurately weighing 100mg of Ezetimibe was transferred into a 100ml volumetric flask and diluted with Ethanol up to the mark. From this pipette put 10ml into a 100ml volumetric flask and diluted with Ethanol up to the mark, from this solution pipette out 2, 4, 6, 8, 10, and 12ml in 10ml individual volumetric flasks and add Ethanol up to the mark, this gives 2, 4, 6, 8, 10, 12 µg/ml concentrations.

Preparation of Sample solution:

The commercially available EZEDOC10 contains 10 mg of Ezetimibe. From this twenty Tablets were weighed and powdered. The Tablet powder equivalent to 100 mg of Ezetimibe was transferred into a 100 ml volumetric flask then it was diluted with the Ethanol and made up to the mark and the solution was filtered through Whatman filter paper NO. 41. From the above solution 10 ml was pipetted out into a 100 ml volumetric flask and the volume was made up to the mark with Ethanol. The final concentration of Ezetimibe was brought to 60µg/ml.

Method validation:

The method is validated according to the ICH guidelines[10,11,12].

RESULTS AND DISCUSSION:

Method: a) Zero-order derivative spectroscopy

Linearity:

The working standard solution was diluted serially with Ethanol to obtain the range of 2-12µg/ml. a calibration curve for Ezetimibe was obtained by measuring the absorbance at the λmax of 234nm and absorbance values are shown in Table.1 and the Calibration graph were presented in Fig.4. Statistical parameters like slope, intercept, coefficient of correlation, and Sandel's sensitivity was determined and presented in Table.2.

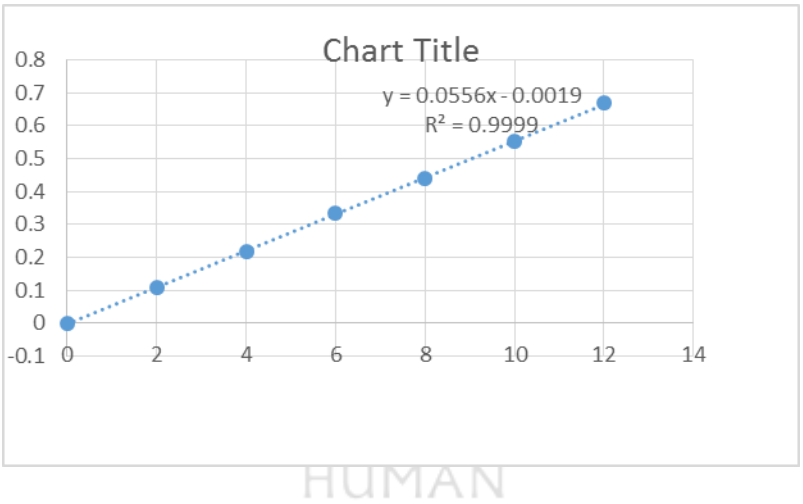

Fig.4: Zero-order calibration curve

Table.1: Results of calibration curve for Ezetimibe at 234 nm by zero-order Spectroscopy.

SL. NO	Concentration in µg/ml.	Mean Absorbance±Standard deviation
1.	2	0.108±0.0015
2.	4	0.219±0.001
3.	6	0.334±0.0037
4.	8	0.44±0.002
5.	10	0.551±0.0095
6.	12	0.668±0.0015

Table no.2: Regression parameters for Ezetimibe by zero-order spectroscopy

Regression Parameters	Ezetimibe
Range	2-12g/ml
Max	234nm
Regression Equation	Y=0.053x+0.0238
Slope (b)	0.0238
Intercept(a)	0.053x
Correlation coefficient (r2)	0.9888
Sandell's Sensitivity	0.0179

Precision:

The precision of the method was studied as intra-day and inter-day precision. Intra-day precision was determined by analyzing the 2, 4, 6, 8,10, and 12 µg/ml concentration three times on same day. Inter-day precision was determined by analyzing the same concentration of solution daily for three days. Precision results are shown in Table.3.

Table.3: Determination of precision results for Ezetimibeat 234 nm by Zero order derivative spectroscopy.

Concentration (µg/ml)	Intra-day Absorbance ±SD**	%RSD	Inter-day Absorbance ±SD**	%RSD
2	0.108±0.0015	1.38	0.107±0.002	1.86
4	0.219±0.001	0.456	0.233±0.0037	1.58
6	0.334±0.0037	1.10	0.347±0.002	0.576
8	0.44±0.002	0.45	0.45±0.003	0.66
10	0.551±0.0095	1.72	0.563±0.004	0.71
12	0.668±0.0015	0.224	0.675±0.0035	0.44

Accuracy:

To assess the accuracy of the proposed method, recovery studies were carried out at three different levels i.e., 50%, 100%, and 150%. In which the formulation concentration was kept constant and varied pure drug concentration. Accuracy results were shown in Table.4.

Table.4: Determination of accuracy results for Ezetimibe by Zero order derivative spectroscopy.

Spiked Levels	Amount of sample (µg/ml)	Amount of standard (µg/ml)	Amount Recovered (µg/ml)	%Recovery ±SD**	%RSD
50	4	2	6.10	101.73±0.0305	0.0299
100	4	4	8.12	101.54±0.0862	0.0848
150	4	6	10.09	100.63±0.045	0.0447

**Average of six determinations

Ruggedness:

Ruggedness was determined by different analysts. The value of %RSD was found to be less than 2 were shown in Table.5.

Table.5: Determination of Ruggedness results for Ezetimibeat 234 nm by Zero order Spectroscopy

Analysts	Analyst-1	Analyst-2
Mean absorbance	0.106	0.109
Standard deviation	0.001	0.002
%RSD	0.943	1.834

Limit of detection and Limit of Quantitation:

The LOD and LOQ of the present method were calculated based on a standard deviation of the Response and slope of the linearity curve. LOD and LOQ values of Ezetimibe were found to be 0.666µg/ml and 6.66µg/ml.

SL.NO	Parameters	Values
1	SD of Intercepts*	0.01108
2	Average of Slopes*	0.0549
3	LOD(3.3×SD of intercepts/average of slopes)	0.666
4	LOQ(10×SD of intercepts/average of slopes)	6.66

*Mean value obtained from 6 calibration curves.

CONCLUSION:

In the present investigation, we have developed a Novel, simple, accurate, and Precise UV-the spectrophotometric method by using Zero order derivative spectroscopy for the routine estimation of Ezetimibe in Bulk and pharmaceutical dosage form, and the methods were validated in terms of linearity, accuracy, precision, ruggedness, and robustness.

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