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# REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR THE ESTIMATION OF EZETIMIBE IN BULK DRUG AND IN PHARMACEUTICAL FORMULATIONS

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**ABSTRACT**

A selective, precise, accurate, linear, rugged and robust for the determination of Ezetimibe tablet dosage form. The method was validated according to ICH and FDA guidelines. Analysis of the drug was performed on Agilent XDB C18 (150mm×4.6 mm, 5µ) column, in an isocratic mode employing di- Sodium Hydrogen Orthophosphate buffer and methanol as the mobile phase in the ratio of 32: 68 v/v UV-Visible detector at 234nm was found to be suitable for detection. Linearity was observed in the range of 20 -100 µg/ml with correlation coefficient of 0.9997. Sensitivity, accuracy, range, precision, robustness, ruggedness, stability, specificity, limit of detection, limit of quantification and system suitability parameters were validated for the developed method. The developed method was successfully applied to estimate the amount of Ezetimibe in pharmaceutical formulations.

**KEYWORDS**

Ezetimibe, HPLC and Chromatography.

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## INTRODUCTION

Ezetimibe [1-3] is an anti-hyperlipidemic agent it is used to lower cholesterol levels. 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 clearance of cholesterol from the blood; this distinct mechanism is complementary to that of HMG-CoA reductase inhibitors.

The Chemical formula is $C_{24}H_{21}F_2NO_3$[1] molecular weight of 409.4[2]. Ezetimibe is a white to almost white powder that is readily soluble in water[4-10]. Analytical methods for Ezetimibe from pharmaceutical dosage form should be developed and validated [10-15]. Several analytical HPLC methods were reported in literature for the quantitative determination of Ezetimibe and its metabolites in human plasma and other biological fluids. Very few achiral and chiral HPLC methods were reported for the detection and quantification of related impurities in the drug substance Ezetimibe, chiral liquid chromatography for the identification of key intermediate and HPLC [20,21] methods. Ezetimibe was also determined for its related substances and assay by HPLC, spectrophotometry and capillary electrophoresis. In the present work the authors have proposed a simple, rapid and accurate reverse phase liquid chromatographic method for the estimation of Ezetimibe. For contributing such a novel cause, through this article, we have tried our best to develop a fast and user-friendly methodology for the estimation of Ezetimibe using reverse phase-HPLC method in bulk drug and pharmaceutical dosage forms [15-20].

## MATERIALS AND METHODS

In the present work, efforts have been made for the estimation of Ezetimibe in bulk and its pharmaceutical dosage forms. Several trials have been made with respect to the mobile phase composition, columns, as well as UV detector's wavelength to develop a suitable and fast method for the analysis of Ezetimibe.

### Materials, Reagents, and Chemicals

Samples of Ezetimibe Standards were obtained from Mylan Labs, HPLC-grade Methanol, and orthophosphoric acid was obtained from S.D fine.

### Equipments

The HPLC instrument used was Shimadzu LC20 With LC Solutions Software was used for data acquisition.

### Chromatographic Conditions

The Chromatographic column, Agilent XDB C18, 150 x 4.6mm, 5µ, column was used as a stationary phase. Mobile phase was prepared by mixing di-Sodium Hydrogen Orthophosphate buffer and methanol as the mobile phase in the ratio of 32: 68 v/v. Injection volume was 10µL. The pump flow rate was 1.mL/min. The eluent was detected at 225nm at 30°C.

### Preparation of Standard Solution

Accurately weighed and transfered 10mg of Ezetimibe working standard into a 100ml volumetric flask add 70 ml of diluents and sonicated to dissolve it completely and made up to the mark with the diluent. Mixed well and filtered through 0.45µm nylon filter and finally10µg/ml solution were prepared.

### Preparation of Sample Solution for Batch Analysis

Commercial samples were used for batch analysis. Five tablets each having strength of 10mg /tablet of Ezetimibe was weighed and transferred in to a 100ml volumetric flask. Added 80mL of diluent and sonicated to dissolve it completely and make volume up to the mark with diluents. Mix well and filter through 0.45µm nylon filter. Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to mark.

### Analytical Method Validation [25-27]

### Specificity of the Method

The terms selectivity and specificity are often used interchangeably. Specificity is the ability of the method to measure the analyte response in the presence of its potential impurities. This parameter was performed to know the retention time of each drug in a mixture and in the sample to understand if any drug-drug interaction or drug-excipient interaction is present.

### System Suitability

System suitability test is used to verify that the resolution and reproducibility of the

chromatographic systems are adequate for the analysis to be done. The tests are based on the fact that the equipment, electronics, samples to be analyzed constitutes an integral system that can be evaluated as such. The limits for system suitability were set for theoretical plates, resolution, and asymmetry.

**Linearity**

Six concentrations of the standard mixture 20, 40, 60, 80 and 100 percent, were injected and chromatogram was recorded. A graph was plotted for the concentration of the corresponding drug versus area. The correlation coefficient for each drug was calculated.

**Accuracy**

To determine the accuracy in sample preparation method of standard additions was made for measuring the recovery of the drugs. To the standard solution known concentrations of the drug (50, 100 and 150 percent) was added. The accuracy was expressed as the percentage of the analytes recovery.

**Method Precision**

It is very important that the method developed should be precise. Six replicates of the sample prepared from the commercial tablets were injected and Assay was calculated to measure the repeatability of retention times and peak area of standard and sample.

**Robustness**

To verify the robustness of the method, the analysis was done under variable flow rates. The flow rate as per the developed method is 1.0mL/min. This has been purposely changed to 0.9mL/min and 1.1mL/min and the chromatogram was obtained.

**Ruggedness**

To test the ruggedness of the method, the analysis was done on different days and different chemists to check for any changes in the chromatograph. The percentage RSD for the retention time and area was calculated.

## RESULTS AND DISCUSSION

After several permutation and combinations, the method reported has been optimized. It is evident from this method that this is a very fast method of analysis compared to the literature available. We have been able to elute the drugs within 7min. In the current days, industries are looking for the methodology which can save sophisticated instruments and chemist's valuable time, and as a result they can release their product analysis report within short time. This is the reason why people are more attracted towards liquid chromatography. In this regard, the current method developed and reported here

**Specificity of the Method**

The retention times of the standard drugs were measured and it was found to be 5.7min. This indicates there is no chromatographic interference between the analytes. The sample solution (pharmaceutical dosage form) was then injected and the chromatogram was obtained. The retention time of the drugs in the dosage form (tablet) was found to be 5.7 minutes, respective HPLC chromatograms are represented in Figures 2-3. There is no specific change in the chromatogram. This indicates that there is no drug Excipient interference and the drugs are properly resolved by this method. Therefore, this is a suitable method for the estimation of Ezetimibe in dosage forms.

**System Suitability**

The suitability of the system was studied by performing the experiment and looking for changes in separation, validated with a holistic approach according to ICH guidelines and details of findings are as below. Retention times and asymmetry of the peaks. Five injections of the standard and two injections of the sample were injected for this purpose. The retention time, theoretical plates values and peak asymmetry were calculated for standard and sample solutions. Results obtained are given in Table No.1.

**Linearity**

The correlation coefficient was calculated, and it was 0.999 which is well within the acceptance criteria. The results are shown in Table No.2. The concentration was found to be proportional to the area, and the response of the detector was determined to be linear over the range of 20 to 100 µg/mL as shown in the Figure No.4.

**Accuracy**

The percentage recovery of the results obtained is listed in Table No.3. The results indicate that the recoveries are well within the acceptance range, therefore, method is accurate and it can be used for the estimation of Ezetimibe.

**Method Precision**

The percentage RSD values for the assays in precision study were calculated. The results as shown in Table No.4 indicate that the method developed is precise.

**Ruggedness**

Data acquired and compared, % RSD of area and RT has been calculated and tabulated in Table No.5. Based on the data, it is evident that the method is Rugged.

**Robustness** Due to deliberate change in the method, no changes were found in the chromatogram, the method developed is robust. The results are shown in Table No.6

**Table No.1: System suitability results**

| S.No | Preparations | Retention time(min) | Theoretical plates | Peak asymmetry | %RSD |
|---|---|---|---|---|---|
| 1 | Standard | 5.5 | 7784 | 1.2 | 0.9 |
| 2 | Sample | 5.5 | 7895 | 1.3 | |

**Table No.2: Linearity results**

| S.No | Ezetimibe (10)mg | |
|---|---|---|
| | Con(µg/ml) | Average area count |
| 1 | 20 | 420760.500 |
| 2 | 40 | 909992.813 |
| 3 | 60 | 1351444.625 |
| 4 | 80 | 1811693.625 |
| 5 | 100 | 2289660.750 |
| 6 | **Slope** | 23198 |
| 7 | **Intercept** | 35140 |
| 8 | **CC** | 0.999 |

**Table No.3: Results for accuracy of the method**

| S.No | Recovery level | % Recovered |
|---|---|---|
| 1 | 50% - Rec 1 | 99.05 |
| 2 | 50% - Rec 2 | 99.97 |
| 3 | 50% - Rec 3 | 99.49 |
| 4 | 100% - Rec 1 | 101.02 |
| 5 | 100% - Rec 2 | 99.23 |
| 6 | 100% - Rec 3 | 98.76 |
| 7 | 120% - Rec 1 | 100.98 |
| 8 | 120% - Rec 1 | 100.59 |
| 9 | 120% - Rec 1 | 100.96 |
| 10 | **Mean** | 100.0105 |
| 11 | **SD** | 0.856538 |
| 12 | **RSD** | 0.856448 |

**Table No.4: Method precision results**

| S.No | Sample No | % Recovered |
|---|---|---|
| 1 | 1 | 98.8868 |
| 2 | 2 | 100.5521 |
| 3 | 3 | 99.40908 |
| 4 | 4 | 99.91919 |
| 5 | 5 | 99.10268 |
| 6 | 6 | 99.21585 |
| 7 | **Mean** | 99.51429 |
| 8 | **SD** | 0.617153 |
| 9 | **RSD** | 0.620165 |

**Table No.5: RSD of the drugs on different days and different analysts**

| S.No | Concentration (µg / ml) | Intra - day precision (Area) | Inter - day precision (Area) |
|---|---|---|---|
| 1 | 60 | 1343671 | 1343671 |
| 2 | 60 | 1336774 | 1336774 |
| 3 | 60 | 1323233 | 1323233 |
| 4 | 60 | 1332944 | 1362944 |
| 5 | 60 | 1350620 | 1350620 |
| 6 | 60 | 1337582 | 1367582 |
| Mean | | 1346092 | 1337471 |
| Std. Dev | | 4682.343 | 9327.897 |
| %RSD. | | 0.347921 | 0.697428 |

**Table No.6: Robustness of the method**

| Condition | Modification | Mean area ± SD | RSD (%) | Mean RT ± SD (min) |
|---|---|---|---|---|
| Mobile phase composition ( v / v) | 60 : 40 | 3705520.64 ± 20330.84 | 0.547 | 7.23 ± 0.029 |
| | 70 : 30 | 427419.711 ± 716.373 | 0.167 | 5.525 ± 0.008 |
| | 80 : 20 | 617254.47 ± 907.74 | 0.147 | 6.517 ± 0.002 |
| Mobile phase $P^H$ | 5.0 | 4690140.69 ± 15354.34 | 0.327 | 6.781 ± 0.033 |
| | 5.5 | 427419.711 ± 716.373 | 0.167 | 5.525 ± 0.008 |
| | 6.0 | 561044.86 ± 5345.65 | 0.952 | 4.213 ± 0.026 |

| | | | | |
|---|---|---|---|---|
| Flow Rate Of Mobile Phase (ml / min) | 0.9 | 2025395.72 ± 15824.86 | 0.782 | 6.221 ± 0.037 |
| | 1.0 | 427419.711 ± 716.373 | 0.167 | 5.525 ± 0.008 |
| | 1.1 | 1653257.56 ± 10568.31 | 0.639 | 5.050 ± 0.045 |

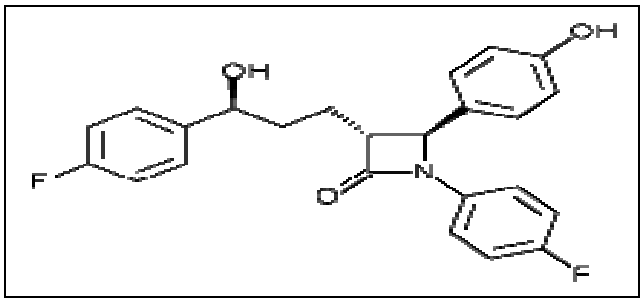


**Figure No.1: Chemical structure of Ezetimibe[7]**

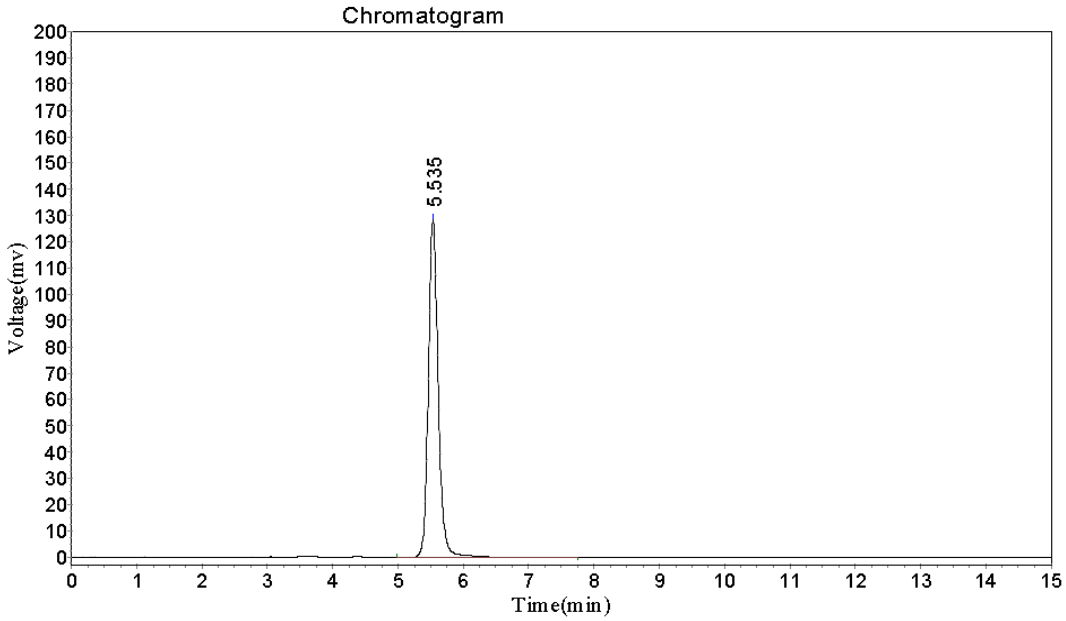


**Figure No.2: Standard chromatogram**

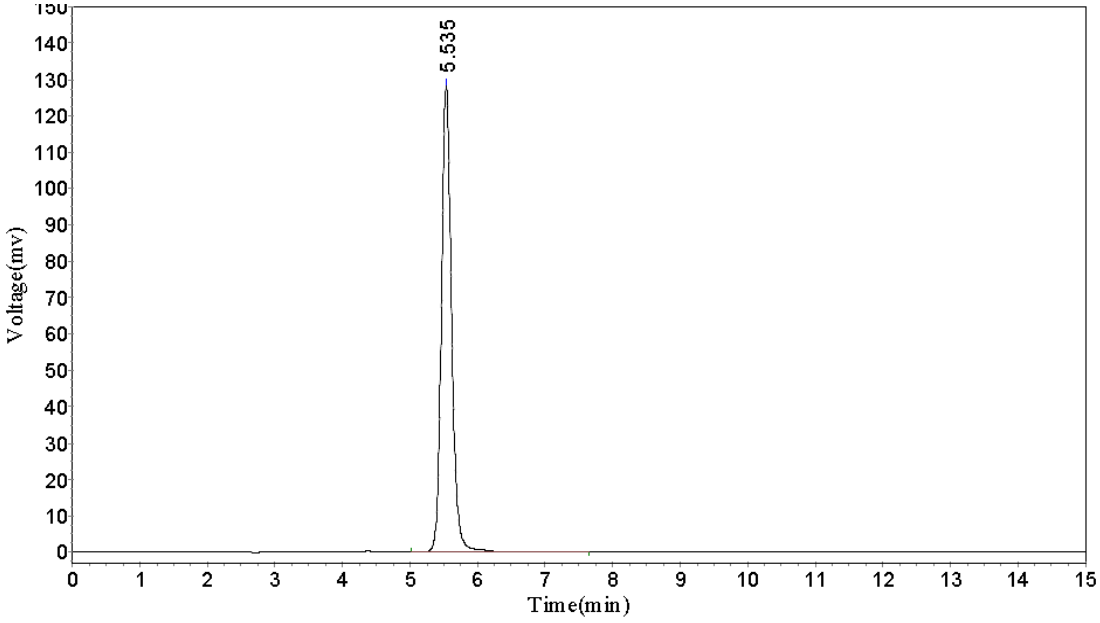


**Figure No.3: Sample chromatogram**

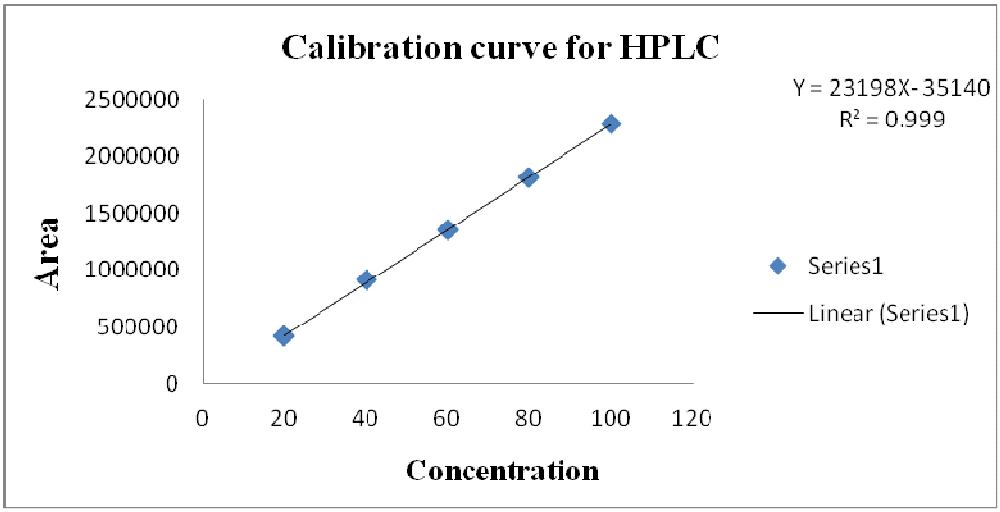


**Figure No.4 : Graphs for linearity of the drugs**

## CONCLUSION

It is concluded from the above study that the current method is fast, reproducible, and simple. By adopting this method one can elute the drugs in 7 minutes. Hence this method is definitely time saving to enable the estimation of Ezetimibe. The proposed method is found to be specific, accurate, precise, linear, robust and rugged.

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