DEVELOP A NEW RP-HPLC METHOD AND VALIDATION FOR QUANTITATIVE DRUG CONTENT DETERMINATION OF EZETIMIBE FROM MARKETED FORMULATED TABLETS

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ABSTRACT

This study has done to develop a cheap, reliable and stable method for the drug content determination of Ezetimibe from its formulated tablets. A binary reversed-phase (C_{18} -Column) high performance liquid chromatograph was used for the analysis. An isocratic method was applied with the mobile phase acetonitrile and water in the ratio of 75:25 (by volume). For detection the Ezetimibe analyte UV-Detector was used at the wave length 230 nm. The average retention time of Ezetimibe was 3.30 minutes. The flow rate was 1.0 mL / min. The method was linear with regression coefficient (R^2) 0.994. The limit of detection (LOD) and limit of quantification (LOQ) were calculated 0.04 and 0.12 $\mu\text{g} / \text{mL}$. Average inter-day and intra-day recovery percentage were computed 98.44 % and 99.62 % with coefficient variation percentage 3.07 and 0.75 %. Recovery results were showed good accuracy of the method. Hence, this method validation was statistically significant.

Keywords: Develop, Validation, RP-HPLC, Ezetimibe.

INTRODUCTION

Quality is the most important attribute of any pharmaceutical product. The quality of a pharmaceutical product was determined by the content of active ingredient using appropriate techniques¹. The Expert Working Group of the International Conference on Harmonization of technical requirements for registration of pharmaceuticals for human use, commonly known as ICH has defined an impurity as “any compound of the medicinal product which is not the chemical entity defined as the active substance or as an excipients in the product”. In the present study has been developed a reversed phase-high performance liquid chromatography method for the analysis of formulated Ezetimibe tablets. The IUPAC name of Ezetimibe is 1-(4-fluorophenyl)-3(R)-[3-(4-fluorophenyl)-3(S)-hydroxypropyl]-4(S)-(4-hydroxyphenyl)-2-zetidin-one².

Ezetimibe is a selective cholesterol absorption inhibitor; which potentially inhibits the intestinal absorption of cholesterol and related phytosterols by the small intestine without affecting absorption of triglycerides, fatty acids, bile acids and fat-soluble vitamins³. The drug is widely used in treatment of hypercholesterolemia and of sitosterolemia. The main aim of this study is to develop a new reversed-phase high performance liquid chromatography method and validation.

Figure 1: Structure of Ezetimibe

MATERIALS AND METHODS

Chemicals and Reagents

99.7 % pure Ezetimibe was purchased from Ranbaxy Pharmaceutical Ltd. Tablets of Ezetimibe in 10 mg were

purchased from Lupin Pharmaceutical Ltd. HPLC grade methanol and water was purchased from Merck India Limited and 0.45 μm nylon membrane filter was supplied by Pall life Sciences, Mumbai, India.

Instrumentation

A reversed phase-high performance liquid chromatograph was used for assay method development and validation. The chromatograph equipped with a binary pump, column thermostat and a UV-detector. The eluent was analyzed using a C_{18} -column at wavelength (λ_{max}) 225 nm with mobile phase acetonitrile and water in the ratio of 75:25 (by volume). Injection volume was used 25 μL . The mobile phase was filtered through 0.45 μm nylon filter membrane followed by sonicate for five minutes prior to use.

Preparation of Stock Solution

Stock solution containing 100 $\mu\text{g} / \text{mL}$ of Ezetimibe was prepared separately in mobile phase. The stock solutions were filtered through a 0.45 μm nylon membrane followed by sonicate for five minutes. Serial dilutions were prepared by appropriate dilution of the stock solutions with mobile phase.

Methods Validation

The methods were validated according to ICH guidelines with respect to linearity, specificity, precision, accuracy, system suitability test, limit of detection (LOD) and limit of quantification (LOQ)⁴.

Linearity

For measuring the linearity serial dilutions of 5, 10, 15, 20 and 25 $\mu\text{g} / \text{mL}$ were prepared from the stock solution of Ezetimibe. The total volume of 10 mL was maintained with mobile phase. These different serial dilutions were filtered through a 0.45 μm nylon membrane and sonicate. The each solution of 25 μL was injected into the column in thrice. The calibration curves were obtained by plotting peak areas versus known concentrations in $\mu\text{g} / \text{mL}$.

Specificity

Specificity was determined with exepient of formulated Ezetimibe tablets. An equivalent weight was taken and solution prepared similarly to the sample solution. The prepared solution was determined as per the described method. After determination was not reported any interference with exepient at the retention time of the peaks of paracetamol and caffeine. Therefore, it is concluded that the method is specific.

Accuracy

The accuracy of the method was determined by recovery method. The recovery was checked at the three theoretical concentrations levels 5, 10 and 15 µg. The chromatograms were recorded and the percentage inter-day and intra-day recovery was calculated.

System Suitability Test

The Reproducibility of sample was checked of the system to measurement of peak area and was carried out using three replicates of same concentration of standard and sample, respectively.

Limit of Detection (LOD) and limit of Quantification (LOQ)

It is a lowest response of the instrument at lowest concentration, so the detectable signal value called as limit of detection and quantifiable noise value called as limit of quantification. It was measured by signal to noise ratio. To determine the limit of detection (LOD) and limit of quantification (LOQ), to prepare three replications⁵⁻⁸ of low

concentrations serial dilutions of Ezetimibe from the standard stock solution.

RESULTS AND DISCUSSION**Selection of Mobile Phase**

It was a basic need of liquid chromatography. To select the mobile phase various composition of mobile phases were checked with water and acetonitrile in different ratio 25:75, 35:65 and 45:55 (by volume) on C₁₈- column at wave length 225 nm and acetonitrile and water 20:80, 30:70 and 40:60 (by volume) on C₁₈- column at wave length 225 nm. The mobile phase acetonitrile and water in the ratio of 75:25 (by volume) was selected. At this mobile phase was obtained suitable retention time and peak area with better resolution.

Chromatographic Conditions

Under experimental conditions the mobile phase acetonitrile and water in the ratio of 75:25 (by volume) was eluate in isocratic mode. The flow rate of elution was 1.0 mL / min. The wave length was set at 225 nm. Ezetimibe depicted a well defined chromatographic separation within a run time of 6.0 min. The retention time of Ezetimibe was 3.30 min.

System Suitability Test

To establish the chromatographic conditions were performed system suitability test (SST) during the development and optimization of the method. The test was performed by injecting the standard mixture in triplicate and the various parameters retention time, area, tailing factor, resolution factor and theoretical plates were computed as reported by USP⁹ and International conference harmonized guidelines (Table 1).

Table 1: Summary of System Suitability Test

Statistics Parameters	Tailing Factor	Retention Time	Area	Resolution	Theoretical Plate
Mean	1.46	3.263	155866.7	0.555	1558.825
SD	0.02	0.047	3.047	0.005	1.89749
CV %	1.50	1.464	0.001	0.986	0.121726
SE	(±) 0.008	(±) 0.019	(±) 1.244	(±) 0.002	(±) 0.774647

SD=standard deviation CV%= coefficient of variation percentage SE=standard error

The parameters tailing factor (T_f), retention time (R_t), area (A), resolution factor (R_f) and theoretical plates (T_p) have less than 10 % coefficient variation percentage (<10). Statistically all these parameters were significant. Therefore the applied method was suitable for the analysis.

Linearity

Linearity was determined by the regression analysis. The calibration curves were plotted between known

concentrations and average peak areas. The method was linear with a correlation co-efficient (R²) 0.994. The result was shown in Table 2.

Figure 2: Calibration Curve of Ezetimibe

Table 2: Statistical Summary of Linearity, Limit of Detection and Limit of Quantification

Parameters	HPLC
Correlation range	5-25 µg / ml
Regression equation	$Y=32603x+3646$
Regression coefficient	$R^2 = 0.994$
Limit of detection (µg / mL)	0.03 µg / ml
Limit of quantification (µg / mL)	0.09 µg / ml

Limits of Detection (LOD) and Limit of Quantification (LOQ)

The limit of detection (LOD) and limit of quantification (LOQ) were calculated using the signal-to-noise-ratio of 3 and 10. The limit of detection (LOD) and limit of quantification (LOQ) values was found 0.03 and 0.09 µg / mL.

Specificity

The specificity of the method was determined by checking the interference with the components from placebo. No interference was observed for any of the components like excipients of both drugs.

Accuracy

The accuracy of the method was computed by determination of recovery for three concentrations (5, 10, 15 µg). The amount of Ezetimibe recovered and then percentage of drug content calculated. The inter-day and intra-day mean recovery of Ezetimibe was found 98.44 % and 99.62 % with less than 10 % coefficient variation percentage (Table 3 and 4). Hence, the results were statistically significant. The recovery results showed that the method was very accurate for quantitative determination of Ezetimibe from formulated tablets.

Table 3: Recovery Study in inter-day

Added pure drug in µg	Recovery in µg	Recovery (%)	Mean	Standard Deviation	CV %
5	4.93	98.62			
10	10.13	101.36	98.44	3.02	3.07
15	14.29	95.32			

Table 4: Recovery Study in intra-day

Added pure drug in µg	Recovery in µg	Recovery (%)	Mean	Standard Deviation	CV %
5	4.98	99.61			
10	10.03	100.39	99.62	0.75	0.75
15	14.83	98.88			

SD = standard deviation CV = coefficient variation percentage

CONCLUSION

The reversed-phase high performance liquid chromatography (RP-HPLC) validation method was developed with isocratic mode. Selected experimental conditions were provided high resolution and repeatability of peaks. For the repeatability of the peaks and retention time the required temperature was 20°C¹⁰. This validated method more reliable to simultaneous determination of Ezetimibe from its formulated tablets. There was no interference from the excipients which used in the tablet formulations and hence the methods are suitable for analysis of formulated tablets. Several results of validation showed that the described reversed-phase high performance liquid chromatography (RP-HPLC) method is simple, linear, precise, accurate and selective. Hence the above method can be recommended for the simultaneous determination of Ezetimibe from formulated tablets.

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