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HPLC METHOD DEVELOPMENT AND VALIDATION FOR EZETIMIBE

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ABSTRACT

A simple, robust, linear analytical method for quantitation of ezetimibe using HPLC was developed and validated. Prepared sample was eluted at 4.9 min by using ACE C18 (250mm X 4.6mm, 5µm) column. 0.1 % Ortho-phosphoric acid and methanol (as 20:80 v/v ratios) was mobile phase for this study. The flow was kept at1.0 mL/min. The peaks were observed at the wavelength of 232nm. All the validation parameters including accuracy and robustness parameters were evaluated in order to validate the method. As per validation results, the method found linear and precise for5 to 30 µg/mL concentrations, accurate at 50% to 150 % of target concentration (10µg/mL) from 5µg/mL to 15 µg/mL, robust at 10% changes at upper and lower side of Organic composition of mobile phases, ± 2nm changes in wavelength selection of 232nm and ± 0.2 mL/min of selected flow rate i.e. of 1.0 mL/min.

Keywords: Analytical Method, HPLC, Validation, Ezetimibe, Mobile Phase

INTRODUCTION

Hyperlipidemia is a medical state related with the lipid metabolism in the body. The lipids move into the blood stream and start to adhere and set up at the wall of blood vessels and arteries. This can lead to conditions like atherosclerosis and increase the risk of cardiovascular diseases. Thus, treatment of hyperlipidemia became important aspect in therapy.

The ezetimibe drug is a Biopharmaceutical Classification System (BCS) class II category drug with $C_{24}H_{21}F_2NO_3$ as molecular formula and 409.4 g/mol molecular weight. It has low aqueous solubility (0.00846 gm/L) as well as poor bioavailability (35%). It targets the jejunal enterocyte brush border uptake, inhibits the cholesterol absorption and act as anti-hyperlipidemic activity **(Figure 1)** **[1, 2]**.

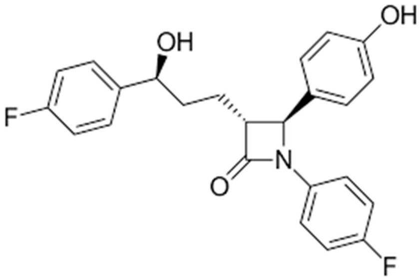

Figure 1: Structure of Ezetimibe

For the quantification and analysis of ezetimibe, there are previously reported analytical methods as per literature available. But this experiment tried to simplify analytical method for quantification with respect to time, man efforts and cost. The objective of this research experiment is to formulate simple, linear, robust, precise, accurate analytical method and validate the same **[3]**.

MATERIALS AND METHODS

Pharmaceutically pure sample of ezetimibe were arranged by Atharva Chemicals, Chiplun (Ratnagiri). The ortho-phosphoric acid and methanol used for analysis were manufactured by Merck. Only analytical and HPLC grade chemicals were employed for the experiment.

Wavelength Selection:

The UV scan for standard solution was carried out at 200 – 600 nm range and the acquired spectrum was examined for maximum absorbance of the drug **[4]**.

Mobile Phase Selection:

The chromatographic analysis was conducted on the ezetimibe standard solution (25 µg/ml). The preliminary study was performed with methanol and acetonitrile in different ratios along with different buffers to get the suitable parameters for the system **[5, 6]**.

Mobile Phase Composition:

The 0.1% ortho-phosphoric acid was mixed well with methanol (20:80 v/v ratios) for solvent composition in HPLC. After filtrating this solution across the 0.45µm membrane, it was kept in ultrasonic water bath for sonication for 10 min to ensure homogenization **[6]**.

Preparation of diluent:

Acetonitrile mixed with HPLC grade distilled water (50:50 v/vratio).

Preparation of Standard Solution:

After precise weighing of 10 mg ezetimibe, added to flask. Then volume was filled upto 20 mL utilizing diluent, sonicated well till drug dissolved completely. The prepared stock solution was of 500 µg/ mL. The dilution of2 mL out of this solution was done upto 20mL. Again out of this 2 mL solution was collected and dilution was made upto 10 ml with diluent and mixed well to prepare standard solution of strength 10µg/ml **[7, 8]**.

Preparation of Chromatogram:

With the help of mobile phase, the HPLC column was stabilized by inoculating prepared solution of ezetimibe (10µg/mL) **[9]**.

Chromatographic parameters:

The chromatographic analysis was carried out by employingAce C18 (250 x 4.6 mm, 5µm) column. 0.1 % Ortho-phosphoric acid and methanol (as 20:80 v/v ratios) was mobile phase for this study. The flow of solvent system was kept at 1.0 mL/min and 232 nm was chosen as scanning wavelength for maximum absorption and peak identification. The sample injector used was 50 µl loops and ambient column temperature was maintained **[10, 11]**.

Validation of method:

The validation for this method for ezetimibe analysis conducted for assessment of linearity, accuracy, precision and robustness. All the guidelines followed were as per ICH **[12, 13]**.

Linearity:

The linearity was detected by analysis of solutions in5-30µg/ml concentration range,and composed from stock solution. This evaluation was conducted in triplicate **[14]**.

Limit of Detection (LOD) and Limit of Quantitation (LOQ) [14]

The LOD and LOQ determined, with equations;

$LOD = 3.3\sigma/ s$ and $LOQ = 10\sigma/ s$,

Where, σ is Standard Deviation, s is Slope of regression equation

Precision:

The intra and inter-day precision evaluations for the developed HPLC method were determined. In the intra-day precision analysis, three separate samples with identical concentrations were examined in a single day. For inter-day precision evaluation, three independent samples of identical concentrations were examined on three consecutive days. The% RSD was determined for precision analysis **[15]**.

Accuracy

To validate the method for accuracy, recovery analysis, the drug penetrated into the placebo sample solution, at around 50, 100 and 150 %levels **[15, 16]**.

RESULTS AND DISCUSSION

The experiment was performed to validate the developed method for quantitation of ezetimibe. The results are given in following tables.

Wavelength Selection:

The 232nm was chosen as wavelength for identification as the drug showed maximum absorbance and peak was observed **(Figure 2)**.

Mobile Phase and Chromatographic Conditions Selection:

After preliminary study, the mobile phase was selected as ortho-phosphoric acid (0.1%) with methanol (20:80 ratios); as it exhibited superior resolution and more suitable peak parameters.

Chromatogram Parameter for Ezetimibe

The **Figure 3** represents the chromatogram of ezetimibe.

The results for parameters for suitability for the drug are enumerated in **Table 1**.

Linearity

Figure 4 represented the calibration curve of ezetimibe.

The results for linearity study are mentioned in **Table 2**.

Range:

From data of linearity found over the range of 5-30 µg/ml.

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

The evaluation for LOD was 0.59µg/ml while that of LOQ was1.79 µg/ml.

Precision:

The evaluation data for precision are mentioned in **Table 3**.

Accuracy

The results of study are mentioned in **Table 4**.

Robustness

The results for robustness analysisas mentioned in **Table 5**.

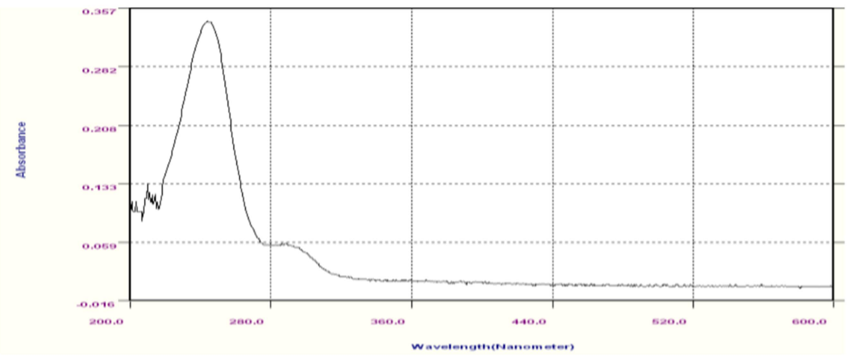

Figure 2: UV-VIS Spectra of Ezetimibe (10 µg/ml)

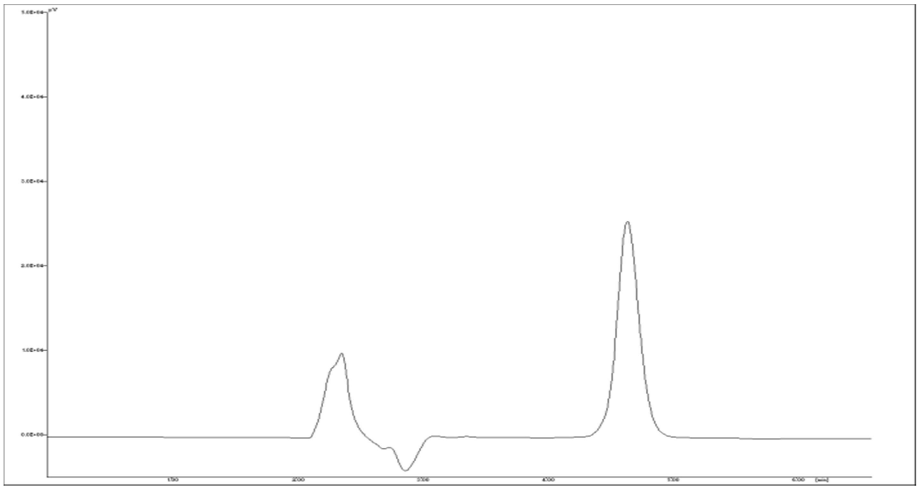

Figure 3: HPLC Chromatogram of Ezetimibe standard solution

Table 1: System suitability parameters for Ezetimibe

Component	RT (Min)	Area	Plates	Asymmetry
Ezetimibe	4.9 ± 0.2	125763	4456	0.98

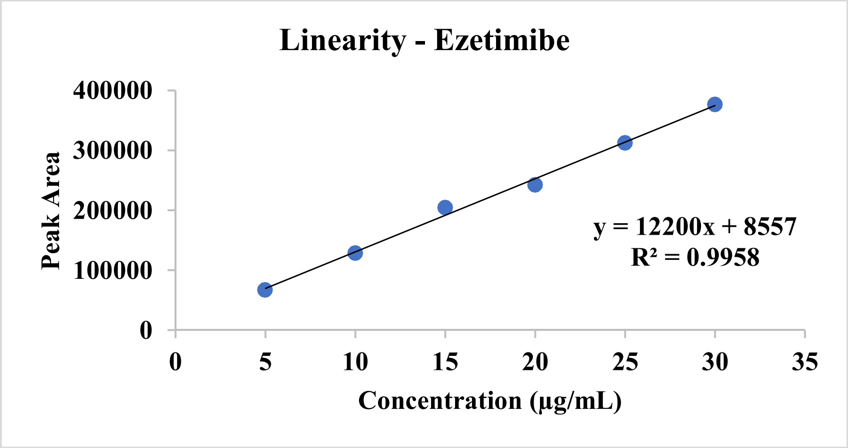

Figure 4: Calibration curve of Ezetimibe

Table 2: Results for Linearity analysis of Ezetimibe

Replicates	Concentrations of Ezetimibe (µg/ml)					
	5	10	15	20	25	30
	Peak Area					
1	67885	127865	204554	240543	310947	375678
2	67345	130092	205345	244563	313679	379756
3	67032	128763	202998	242864	312456	374564
Mean	67421	128907	204299	242657	312361	376666
Std. Dev.	431.5	1120.4	1194.1	2018.0	1368.5	2733.4
%RSD	0.64	0.87	0.58	0.83	0.44	0.73

Table 3: Evaluation of precision analysis of ezetimibe

Replicates	Intraday Precision			Inter day Precision		
	Conc. (µg/ml)					
	5	10	30	5	10	30
1	68388	129934	373458	67945	128564	370945
2	69439	130045	373797	69564	127634	369826
3	68894	132982	368693	68354	129455	371325
Mean	68907	130987	371983	68621	128551	370699
SD	525.6	1728.6	2854.0	841.9	910.6	779.3
%RSD	0.76	1.32	0.77	1.23	0.71	0.21

Table 4: Recovery study analysis of Ezetimibe

Level	Concentration of solution after spiking of Ezetimibe (µg/ml)	Area	Recovery Amount (µg/ml)	% Recovery	% Recovery
50%	5	64554	5.09	101.7	100.4
		63734	5.02	100.4	
		62904	4.96	99.1	
100%	10	126544	9.97	99.7	100.4
		127864	10.07	100.7	
		128047	10.09	100.9	
150%	15	189095	14.90	99.3	100.3
		190657	15.03	100.2	
		193005	15.21	101.4	

Table 5: Data for Robustness analysis

% RSD found For Robustness analysis(peak area)								
MP Composition (± 10% Organic Composition)			Detection Wavelength (± 2 Nm)			Flow Rate (± 0.2 Ml/Min)		
20:72	20:80	20:88	230	232	234	0.8	1.0	1.2
1.2	0.8	1.1	1.7	0.8	1.2	1.3	1.1	1.5

CONCLUSION

In the current experimental analysis, the development of HPLC method for ezetimibe was carried out and validated the same. The HPLC method found simple, rapid, economic method for estimation of ezetimibe. Thus, it is feasible to implement this method for routine laboratory analysis of the ezetimibe. The analytical parameters and mobile used for the method development provided better results. The method reproduced accurate and precise results under varied chromatographic conditions. All the ICH guidelines were followed during this development and validation. This method can be used in laboratories for estimation of ezetimibe from sample or dosage forms.

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