

Spectrophotometric Determination of Ezetimibe

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Abstract: Two simple, sensitive, selective and accurate spectrophotometric methods (Method A and Method B) for the determination of ezetimibe in bulk drug and pharmaceutical formulations (tablets) have been described. Method A and B are based on the redox/complex formation reaction of drug with 1,10-phenanthroline and hexacyanoferrate(III) in presence of ferric chloride to form coloured chromogens exhibiting λ_{max} at 510 and 740 nm respectively. The results of analysis for the two methods have been validated statistically and by recovery studies. The results are compared with those obtained using UV spectrophotometric method in alcohol at 231.7 nm.

Keywords : Spectrophotometric determination, Assay, Ezetimibe

Introduction

Ezetimibe (EZM) is a selective absorption inhibitor that effectively blocks intestinal absorption of dietary and biliary cholesterol¹. Chemically known as 1-(4-fluoro phenyl)-3(R)-[3-(4-fluoro phenyl)-3(S)-hydroxy propyl]-4(S)-(4-hydroxy phenyl)-2-azetidinone. Literature survey reveals that spectrophotometry², high performance liquid chromatography³ and liquid chromatography-tandem mass spectroscopy⁴ methods have been reported for the estimation of EZM in pharmaceutical formulations and biological fluids. Although spectrophotometric methods are the instrumental methods of choice commonly used in industrial laboratories, no colorimetric method has been reported so far for the determination of ezetimibe. Therefore, the need for a fast, low cost and selective method is obvious, especially for routine quality control analysis of pharmaceutical products containing ezetimibe. Two spectrophotometric methods A and B, based on the redox/complex formation reaction of EZM with the reagents such as 1,10-phenanthroline (*o*-phen) and hexacyanoferrate [Fe(CN)₆] in presence of ferric chloride[Fe(III)] have been developed.

Experimental

Spectral and absorbance measurements were made with digital Elico UV-Vis spectrophotometer SL 159 and pH measurements were made with Digisun Electronics digital pH meter model DI-707.

Reagents

All the chemicals and reagents were of analytical grade and the freshly prepared solutions were always used in the investigations.

Aqueous solutions of 1.10×10^{-3} M *o*-phen, 2.0×10^{-2} M *o*-phosphoric acid and 3.32×10^{-3} M Fe(III) were prepared for method A. Aqueous solutions of 3.02×10^{-3} M potassium ferricyanide, 3.32×10^{-3} M Fe(III) and 1N hydrochloric acid (HCl) were prepared for method B.

Working standard drug solution

Ezetimibe (~100 mg) was accurately weighed and dissolved in minimum amount of 0.1N sodium hydroxide (0.1N NaOH) followed by dilution to 100 mL with distilled water in standard flask. A portion of stock solution was diluted stepwise with the distilled water to obtain the working standard ezetimibe solution of 100 µg/mL (Methods A&B).

Assay

Method A

Aliquots (0.5-3.0 mL, 100 µg/mL) of the standard EZM solution were transferred into a series of 25 mL calibrated flasks and then solutions of Fe (III) (1.5 mL) and *o*-phen (2.0 mL) were added successively. The total volume in each flask was brought to 10.0 mL with distilled water and heated for 30 min in a boiling water bath. After cooling to room temperature, 2.0 mL of *o*-phosphoric acid was added, the volume in each flask was made up to the mark with distilled water. The absorbances of the coloured complex solution was measured after 5 min at 510 nm against a reagent blank prepared similarly. The content of the drug was computed from the calibration graph.

Method B

Into a series of 10 mL calibrated tubes, aliquots of standard EZM solution (0.5-2.5 mL, 100 µg/mL) were transferred and 1 mL of 3.32×10^{-3} M ferric chloride solution was added. The tubes were stoppered immediately and shaken well for 5 min. Then 0.5 mL of 3.02×10^{-3} M potassium ferricyanide solution was added into each tube and was closed with lids immediately. After 5 min. 1 mL of HCl was added and the final volume was made up to 10 mL with distilled water. The absorbance of the solution in each tube was measured immediately at 740 nm against a similar reagent blank. The amount of the drug was calculated from its calibration curve.

The method has also been applied to pharmaceutical formulations. An accurately weighed amount of tablet powder equivalent to 100 mg of EZM was extracted with methanol (4x15 mL) and filtered. The combined filtrate was evaporated to dryness and the residue was dissolved in the same solvent to get a concentration of 1 mg/mL. The solution was further diluted stepwise with methanol to get working standard solutions for method A. For method B the residue obtained by evaporating methanol extract in separate bulk was dissolved in minimum volume of 0.1N NaOH and subsequently the volume was brought to 100 mL with the distilled water to get working standard solution.

Results and Discussion

Ezetimibe exhibits reducing property due to the presence of phenolic hydroxyl moiety enabled the use of its oxidation reaction followed by complex formation of drug with 1,10-phenanthroline in presence of ferric chloride to produce pink coloured chromogen exhibiting λ_{\max} at 510 nm. Method B is based on the oxidation of EZM by excess ferric salt (Fe(III) or Fe^{3+}) and reduced form Fe(III) (*i.e.* Fe(II) or Fe^{2+}) which subsequently reacts with potassium ferricyanide to give ferrous ferricyanide λ_{\max} at 740 nm.

The optical characteristics such as Beer's law limits, absorption maxima, molar absorptivity, Sandell's sensitivity are presented in Table 1. The regression analysis using the method of least squares was made for the slope (b), intercept (a) and correlation (R) obtained from different concentrations and the results are summarized in Table 1. The percent relative standard deviation and percent range of errors (0.05 level and 0.01 confidence limits) were calculated for the two methods and the results are given in Table 1. The optimum conditions for the colour development were established by varying the parameters one at a time in each method, keeping the others fixed and observing the effect produced on the absorbance of the coloured species. The values obtained for the determination of ezetimibe in tablets by the proposed and UV methods are compared in Table 2. To evaluate the validity and reproducibility of the method, known amounts of pure drug were added to previously analysed pharmaceutical preparations and the mixtures were analyzed by the proposed methods. The percent recoveries are given in Table 2. These studies revealed that the common excipients are usually present in the dosage forms did not interference at their regularity added levels. The results indicate that the methods are accurate, precise and reproducible and are applicable to various formulations of ezetimibe.

Table 1. Optical characteristics, precision, accuracy of the methods proposed in the determination of ezetimibe.

S.No.	Optical Characteristics	Method A	Method B
1.	λ_{\max} , nm	510	740
2.	Beer's Law Limits, $\mu\text{g/mL}$	2.0-12.0	5.5-28.0
3.	Molar absorptivity, $\text{L mol}^{-1}\text{cm}^{-1}$	1.45×10^4	5.51×10^4
4.	Correlation coefficient (r)	0.9999	0.9997
5.	Sandell's sensitivity, $\mu\text{g/cm}^2/0.001$ absorbance unit	0.092	0.176
6.	Regression Equation ($y = a+bC$)		
	(i) Slope (b)	0.036	0.013
	(ii) Intercept (a)	-0.0005	0.0028
7.	Relative Standard Deviation *	0.868	0.935
8.	% of range error (confidence limit)		
	(i) 0.05 level	0.911	0.984
	(ii) 0.01 level	1.429	1.539

*Average of six determinations considered..

Table 2. Determination of ezetimibe in pharmaceutical formulations.

Sample*	Labelled amount, mg	Amount found by proposed methods**		Ref.Method (UV method)	% Recovery by proposed methods***	
		Method A	Method B		Method A	Method B
Tab I	10	10.01±0.065	9.98±0.052	9.96±0.034	100.02±0.65	99.82±0.52
		F = 3.65 t = 1.75	F = 2.34 t = 0.80			
Tab II	10	9.96±0.092	10.02±0.069	9.94±0.073	99.64±0.92	100.02±0.68
		F = 1.58 t = 0.42	F = 1.12 t = 1.95			
Tab III	10	9.92±0.063	9.97±0.14	9.98±0.125	99.25±0.62	99.77±1.40
		F = 1.44 t = 1.12	F = 1.25 t = 0.13			
Tab IV	10	9.93±0.27	9.89±0.19	10.04±0.36	99.33±0.62	98.91±1.90
		F = 1.77 t = 0.60	F = 3.59 t = 0.94			

* Tablets from four different pharmaceutical companies.

** Average ± standard deviation of six determinations, the *t*- and *F*-test values refer to comparison of the proposed method with the reference method. Theoretical values at 95% confidence limit, *F* = 5.05, *t* = 2.228

*** Recovery of 10 mg added to the preanalysed pharmaceutical formulations (average of three determinations).

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