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SPECTROPHOTOMETRIC DETERMINATION OF EZETIMIBE USING MBTH REAGENT IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT
Simple, precise, accurate and sensitive extractive method has been developed for the quantitative estimation of ezetimibe in bulk drugs and pharmaceutical formulations (tablets). The method is based on reaction of oxidative coupling of ezetimibe with 3-Methyl-2-Benzthiazolinone hydrochloride (MBTH) to form green colored product. Ezetimibe at its λmax 633 nm shows linearity in the concentration range of 2-8µg/ml. The relative standard deviations of 0.404% were obtained. Linear relationships with good correlation coefficients 0.999 were found between absorbance and the corresponding concentrations of the drug. The reliability and performance of the proposed methods was validated statistically the percentage recovery ranged from 96.6 and 105% respectively. The results of analysis for this method have been validated statistically and by recovery studies.
KEYWORDS: spectrophotometric, oxidative coupling, 3-Methyl-2-Benzthiazolinone hydrochloride (MBTH), ezetimibe, ferric chloride.

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INTRODUCTION

Ezetimibe (EZTB),(3R,4S)–1-(4-fluorophenyl)-3-[(3S)-(4-fluorophenyl)-3-hydroxypropyl]-4-4-hydroxyphenyl)-2-azetidinone[1] (Figure 1) is a selective cholesterol absorption inhibitor, which potently inhibits the absorption of biliary and dietary cholesterol from the small intestine without affecting the absorption of fat-soluble vitamins, triglyceride or bile acids. Ezetimibe reduces the small intestinal enterocyte uptake and absorption of cholesterol that keeps the cholesterol in the intestinal lumen for excretion. Ezetimibe is rapidly absorbed and primarily metabolized in the small intestine and liver to its glucuronide, both of which undergo enterohepatic recycling in humans. Ezetimibe complements the lipid lowering effects of other therapies, such as statins. Clinical studies have shown that co-administration of ezetimibe with statins could provide significant reductions in both the low-density lipoproteins (LDL) and the total cholesterol with slight increase in the high-density lipoproteins (HDL). Also co-administration of ezetimibe with statins could significantly reduce the risk of coronary heart disease (CHD) events in patients with hypercholesterolemia.

Ezetimibe was determined with or without combination of several drugs by HPLC and spectrophotometrically[2-16]. Literature method has been reported yet for the analysis of Ezetimibe in bulk and dosage form.

Although spectrophotometric methods are the instrumental methods of choice commonly used in industrial laboratories, no colorimetric method with the reagent using MBTH 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. The spectrophotometric method was, based on the redox/complex formation reaction of ezetimibe with the reagent 3-Methyl-2-Benzthiazolinone hydrochloride (MBTH) have been developed.

MATERIAL AND METHODS

Apparatus

A Shimadzu UV-visible spectrophotometer model 1800 with 1 cm matched quartz cell was used for the absorbance measurements. Systonics electronic balance was used for weighing the samples.

Reagents and solutions

All employed chemicals were of analytical grade and high-purified water was used throughout. Ezetimibe pure sample was obtained as a gift sample from Lupin Ltd, Mumbai, India. The formulation was purchased from the local market.

Standard solutions

Standard ezetimibe stock solution (1000 µg/ml) was prepared by dissolving 100mg of drug in 100ml of methanol. Working solutions of the drug were prepared by dilution of the stock solution. The marketed tablet form of ezetimibe used in the determination was EZEDOC10 with a labelled strength of 10 mg and manufactured by Lupin Ltd, Mumbai, India.

Reagents

3-Methyl-2-Benzthiazolinone hydrochloride (MBTH) 0.5 %(w/v)

0.5 g of MBTH reagent was accurately weighed transferred into a 100 ml calibrated flask, dissolved in distilled water, and make up the volume up to the mark to obtain a solution of 0.5% (w/v).

Ferric chloride (1%)

Freshly prepared was prepared by dissolving 1 g of ferric chloride in 100 ml of distilled water

General recommended procedures

Procedure for calibration graph

Standard solutions of ezetimibe in methanol, having final concentrations in the range of 3-7 µg/ml were transferred into a series of 10 ml volumetric flasks. To each 2 ml of MBTH, 2 ml of ferric chloride was added and the volume was made up to mark with methanol and allowed to stand for 20 minutes. The contents were diluted up to 10 ml with methanol. The absorbance of each solution was measured at 633 nm against the reagent blank. The colored species was stable for 2 h and the amount of drug in the sample was computed from its calibration curve and absorption spectra are represented in **Figure 2** and **Figure 3**.

Procedure for pharmaceutical formulations

Ten tablets were weighed and their contents are mixed thoroughly. An accurately weighed portion of powder equivalent to the 10 mg of ezetimibe was weighed into a 100 ml volumetric flask containing about 50 ml of methanol. It was shaken thoroughly for about 5-10 minutes, filter thoroughly with whatman filter paper to remove insoluble matter and diluted to the mark with methanol to prepare 1000 µg/ml solution. An aliquot of this solution was diluted with methanol to obtain a concentration of 5 µg/ml. Then to that solution 2 ml of 0.5% MBTH, 2 ml of 1% $FeCl_3$ is added. The mixture was then gently shaken and the appearance of green color occurs. The contents were diluted up to 10 ml with methanol.

RESULTS AND DISCUSSION

In the present method, the drug reacts with MBTH in the presence of $FeCl_3$ to give a green colored product. Actually, this is an iron catalyzed oxidative coupling reaction of MBTH with the drug. Under the reaction conditions, on oxidation, MBTH loses two electrons and one proton forming an electrophilic intermediate, which is the active coupling species. This intermediate undergoes electrophilic substitution with the drug to form the colored product. The colored products were found to be stable for 4 hours, at room temperature. Reproducible results were obtained in the temperature range of 20–40 °C. The reagent blank has negligible absorbance in the range used for detection of the ezetimibe. Beer's law is obeyed in the range of 2-8 µg/ml for ezetimibe.

In the present investigation, MBTH reagent forms colored complex with ezetimibe and the absorbances were measured at 633 nm respectively. An oxidative coupling reaction takes place with ezetimibe with MBTH reagent. Therefore, the present study was devoted to explore MBTH reagent as oxidative coupling reagent for the determination of ezetimibe in pure and pharmaceutical dosage forms.

Optimization of the spectrophotometric conditions was intended to take into account the various goals of method development. Analytical conditions were optimized via a number of preliminary experiments. The optimum conditions for the reaction were carefully studied. Maximum absorption at 633 nm was obtained immediately upon using 2 ml of 1% $FeCl_3$ and 2 ml of 0.5% MBTH at ambient temperature and the product remained stable for 4 h.

Optimization of parameter

The optimum concentration and volume were selected on the basis of their ability to give maximum absorbance. Different concentrations and different volumes were tried for all the reagents, by varying the parameters at a time. In this method it was found that optimum concentration of MBTH reagent was 0.5%w/v and optimum concentration of $FeCl_3$ was 1% w/v. The optimum volume was found to be 2 ml for MBTH and that of $FeCl_3$ was 2 ml.

Stability of the Chromogen

The reaction between ezetimibe and MBTH completed within 20 minutes. The green colour developed was found to be stable for long period and showed no change in the colour intensity with time. This allowed the method to be followed for the intra-day studies.

Quantification

The limits of the Beer's law, the molar absorptivity and the Sandell's sensitivity values were evaluated and are given in **Table 1**. Regression analyses of the Beer's law plots at their respective λmax values revealed a good correlation. Graphs of absorbance versus concentration showed zero intercept, and are described by the regression equation, $Y = bX + c$ (where Y is the absorbance of a 1 cm layer, b is the slope, c is the intercept and X is the concentration of the drug in µg/ml) obtained by the least-squares method. The results are summarized in **Table 1**.

Validation of the method

The validity of the method for the assay of ezetimibe examined by determining the precision and accuracy. These were determined by analyzing six replicates of the drug within the Beer's law limits. The low values of the relative standard deviation (R.S.D.) indicate good precision of the methods. To study the accuracy of the methods, recovery studies were carried out by the standard calibration curve method. For this, known quantities of pure ezetimibe were mixed with definite amounts of pre-analyzed formulations and the mixtures were analyzed as before. The total amount of the drug was then determined and the amount of the added drug was calculated by difference. The results are given in **Table 2**. The average percent recoveries obtained were quantitative indicating good accuracy of the methods.

Linearity

To establish linearity of the proposed methods, a series of solutions of ezetimibe for 2-8 µg/ml, were prepared from the stock solutions and analyzed. Least square regression analysis was performed on the obtained data.

Precision

The precision of the proposed methods was ascertained by actual determination of six replicates of fixed concentration of the drug within the Beer's range and finding out the absorbance by the proposed method.

Accuracy

The accuracy of the method is the closeness of the measured value to the true value for the sample. To determine the accuracy of the proposed method, different levels of drug concentrations three serial dilutions were prepared from independent stock solutions and analyzed. Accuracy was assessed as the percentage relative error and mean % recovery (**Table 3**).

Ruggedness

To ascertain the ruggedness of the methods, four replicate determinations at different concentration levels of the drugs were carried out. The within-day RSD values were less than 1% and this indicate that the proposed method has reasonable ruggedness.

Limit of detection (LOD) and limit of quantitation (LOQ)

The LOD and LOQ for ezetimibe by the proposed method were determined using calibration standards. LOD and LOQ were calculated as 3.3 σ/S and 10 σ/S, respectively, Where S is the slope of the calibration curve and σ is the standard deviation of y-intercept of regression equation.

CONCLUSION

The reagents utilized in the proposed methods are cheap, readily available and the procedures do not involve any critical reaction conditions or tedious sample preparation. Moreover, the methods are free from interference by common additives and excipients. The wide applicability of the new procedures for routine quality control was well established by the assay of ezetimibe in pure form and in pharmaceutical preparations.

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Table 1: Optical characteristics

Parameter	Values
λ_{max}/ nm	633 nm
Beers law limits (µg/ml)	2-8
Molar absorptivity (1 /mol/cm)	409.43×10^3
Correlation coefficient (R)	0.999
Sandell's sensitivity(ng cm^{-2})	0.0178
Regression equation (y)	y = 0.062x+0.003
Slope, *b*	0.062
Intercept, *c*	0.003
Relative standard deviation%	0.404
% Range of error (95% confidence limits)	0.165
Limit of detection (µg/ml)	0.093
Limit of quantification(µg/ml)	0.258

$Y = bX + c$, where X is the concentration of drug in µg/ml; Average of six determinations.

Table 2: Recovery studies for ezetimibe

S.No	Standard ezetimibe (ml)	Standard ezetimibe (µg)	Sample ezetimibe (ml)	Sample ezetimibe (µg)	Absorbance at 633nm	Amount of Ezetimibe from std. graph	Recovery of std (mg)	%Recovery
1	0.2	2	0.2	2	0.309	4.1	2.01	100.6%
2	0.3	3	0.2	2	0.448	5.97	2.97	99%
3	0.4	4	0.2	2	0.607	8.05	4.15	101%

Table 3: Evaluation of accuracy and precision

Drug	S.No	Label Claim (mg)	Amount found*	% Purity*	Average (%)	S.D	R.S.D[a]	RSD[b]	S.E.M
Ezetimibe	1	10	9.98	99.8	99.31	0.0453	0.587	0.632	0.13
	2		9.95	99.5					
	3		9.85	98.5					
	4		9.93	99.3					
	5		9.92	99.2					
	6		9.96	99.6					

SD. Standard deviation; SEM. Standard error of mean; RSD.relative standard deviation;
a. intraday precision, b. interday precision.

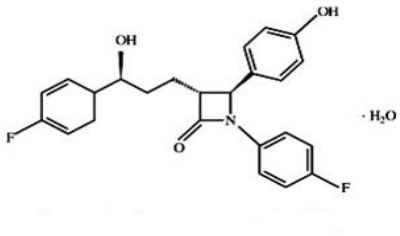

Figure 1: Chemical structure of Ezetimibe

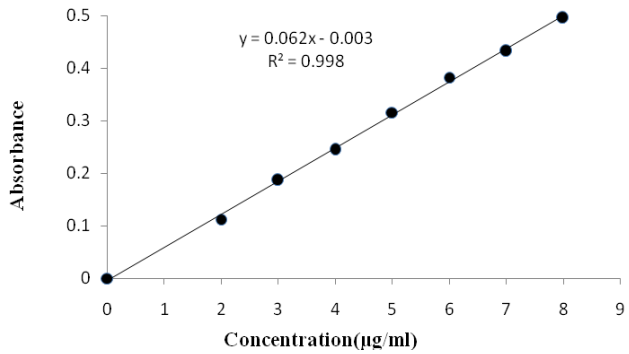

Figure 2: Calibration Graph of Ezetimibe Con=2-8µg/ml

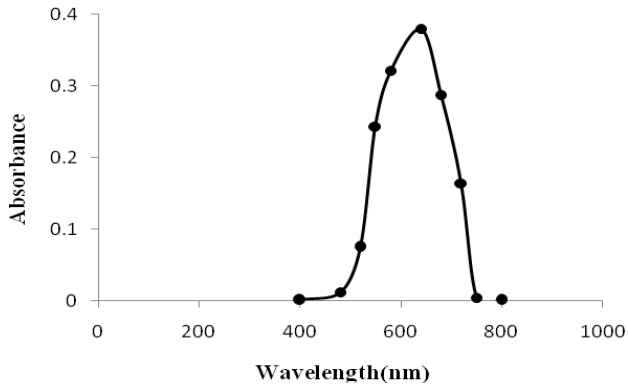

Figure 3: Absorption spectra of MBTH with ezetimibe against the reagent blank

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