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Reversed Phase High Pressure Liquid Chromatographic Analysis of Ezetamibe in Pure and in Pharmaceutical Dosage Forms

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A rapid, simple and precise reverse phase high performance liquid chromatography (RP-HPLC) method was developed for the estimation of ezetamibe in pure form as well as in pharmaceutical dosage forms. The quantification was carried out using a BDSHypersil C-18 column 250 mm × 4.6 mm i.d. 5 μm particle size in isocratic mode, with mobile phase comprising phosphate buffer and acetonitrile in the ratio of 45 : 55 (v/v) at a flow rate of 1.0 mL /min. The eluent was monitored at 236 nm. The retention time was 4.118 min. The calibration curve was linear in the concentration range of 25–300 μg/mL and the percentage recovery ranged from 99.95–100.03.

Key Words: RP-HPLC, Ezetamibe tablets.

INTRODUCTION

Ezetamibe [EZM][1] is a cholesterol reducing agent. Chemically[2,3] EZM is 2-azetidinone, 1-(4-fluorophenyl)-3-[(3s)-3-(4-fluorophenyl)-3-hydroxypropyl]-4-(4-hydroxyphenyl). Literature survey reveals that no HPLC methods were reported for the estimation of ezetamibe. The proposed method was simple, rapid, sensitive, highly accurate and precise for the estimation of ezetamibe in bulk as well as in pharmaceutical dosage forms.

EXPERIMENTAL

An isocratic high performance liquid chromatograph using Waters Alliance 2695 separations module equipped with 600e controller pump and 776 auto sampler. Detection was carried out with a model 2487 dual λ absorbance detector. The chromatographic column used was a 250 mm × 4.6 mm hypersil BDS C-18 with 5 μm particles. The system was equipped with Empower software on Pentium computer. The column was kept at 30°C using a Waters column heater.

Ezetamibe was a gift sample received from Dr. Reddy's laboratories, Hyderabad. HPLC grade acetonitrile and methanol from E. Merck (India) Ltd., Mumbai, potassium dihydrogen orthophosphate of AR grade (rankem Ltd.), orthophosphoric acid (Qualigens) and milli-Q water were used for preparing the mobile phase.

pH 3.0 buffer preparation: 1.36 g of potassium dihydrogen orthophosphate was diluted to 1 L and adjusted to pH 3.0 with dilute orthophosphoric acid.

Chromatographic conditions: The mobile phase used was potassium dihydrogen orthophosphate buffer in the ratio of 45 : 55 (v/v). Buffer and acetonitrile were filtered through 0.45-μ membrane filter and sonicated before use. The mobile phase was pumped from solvent reservoir in the ratio of 45 : 55 to the column at a flow rate of 1 mL /min. The run time was set at 7 min. The column was maintained at 30°C and the volume of each injection was 10 μL. Prior to injecting solutions, the column was equilibrated for at least 30 min with mobile phase flow through the system. The detector sensitivity was set at 0.0001 A.U.F.S. and eluent monitored at 236 nm.

Procedure: About 100 mg of pure sample of ezetamibe was weighed accurately and transferred to a 100 mL volumetric flask and dissolved in 75 mL of methanol. The solution was sonicated for 20 min and then the volume made up with a further quantity of methanol to get 1 mg/mL solution. Subsequent dilutions of this solution ranging from 25–300 μg/mL were made in 10 mL volumetric flasks. The solutions prepared as above were filtered through 0.45 μm membrane filter and then 10 μL of filtrate was injected each time into the column at a flow rate of 1 mL /min. Each concentration was injected six times into the chromatogram and the corresponding chromatograms were obtained. Evaluation of the drug was performed at 236 nm. From the chromatograms, the retention time and mean peak area was recorded for all the concentrations. The plot of peak area *vs.* the respective concentration gives the calibration curve. The regression of drug concentration over the peak area was computed using the least squares method of analysis. This regression equation was used to estimate the amount of ezetamibe in pharmaceutical formulations.

Estimation of ezetamibe in tablet dosage forms: Two commercial brands of tablets were chosen for testing the suitability of the proposed method to estimate ezetamibe in tablet dosage forms. For this, 20 tablets were weighed and powdered. Accurately weighed portion of tablet powder equivalent to 100 mg was taken in a 100 mL volumetric flask and 50 mL methanol was added, shaken well and allowed to stand for 15 min with intermittent sonication to ensure complete solubility of the drug. The mixture was then thoroughly mixed and made up to the mark with methanol and filtered through a 0.45-μ membrane filter. From the filtrate, different aliquots were taken in separate 10 mL volumetric flasks. The contents of the flask were made up to volume with methanol and mixed well. Each of these solutions (10 μL) was then injected into the column. All the determinations were conducted five times from the peak areas. The drug content in the tablets was quantified using the regression equation obtained from the pure sample.

RESULTS AND DISCUSSION

The development of an analytical method for the determination of drugs by HPLC has received considerable attention in recent years because of their importance in quality control of drug and drug products. The aim of this study

was to develop a simple, rapid, accurate and precise HPLC method for the analysis of ezetamibe in bulk and tablet dosage forms using most commonly employed RP C-18 column with UV detection.

The run time of the method was set at 7 min and ezetamibe appeared on the chromatogram at 4.118 min. This indicates that the present HPLC method is rapid, which in turn shows that the method consumes less volume of HPLC solvents. When the same drug solution was injected 6 times, the retention time of the drug was same.

The peak areas from such different concentrations set up as above were calculated and are shown in Table-1. A good linear relationship (r = 0.9999) was observed between the concentration of ezetamibe and the respective peak area. The regression curve was constructed by least squares method and its mathematical expression was y = 28994.72x + 4527.41 (where y is the peak area and x is the concentration of ezetamibe). This regression equation was used to estimate the amount of ezetamibe in pharmaceutical dosage forms and in validation.

TABLE-1
CALIBRATION OF THE PROPOSED METHOD

Drug concentration (μg/mL)	Peak area*	CV (%)
25	725206	1.42
50	1450248	2.42
100	2901486	0.47
150	4352326	1.21
200	5803004	0.47
250	7253400	1.52
300	8704306	0.92

*Mean of six determinations.
Regression equation from 25–300 μg/mL
Y = 28994.72X + 4527.41 (r = 0.9999)

To ensure the reliability and accuracy of the method, recovery studies were carried out by mixing a known quantity of drug solution with preanalyzed sample and the contents were reanalyzed by the proposed method. The values are shown in Table-2. About 99.8% of ezetamibe could be recovered from the preanalyzed samples indicating the high accuracy of the proposed HPLC method.

The HPLC method, developed in the present study, has also been used to quantify ezetamibe in tablet dosage forms. Ezetamibe tablets (conitaining 10 mg of the drug) were quantified using the proposed analytical method and the results are given in Table-3. No interfering peaks were found in the chromatogram indicating that the tablet excipients did not interfere with the estimation of drug by proposed HPLC method. The tablets were found to contain 99.96–100.5% of the drug. A known amount of drug solution was added to the sample of tablet dosage form and subjected to estimation of drug by proposed method. There was

a high recovery of ezetamibe (99.95–100.03%) indicating that the proposed procedure for determination of ezetamibe in tablet dosage form is highly accurate.

TABLE-2
RESULTS OF RECOVERY STUDY

Amount of drug added (μg)	Recovery from drug solution		Recovery from tablet formulation	
	Mean amount found (n = 5)	Mean % recovery	Mean amount found (n = 5)	Mean % recovery
100	100.20	100.20	100.30	100.30
200	199.60	99.80	199.99	99.95
300	300.30	100.10	300.10	100.03

TABLE-3
ASSAY OF EZETAMIBE IN TABLET DOSAGE FORMS

Brand	Labelled amount of drug (mg)	Mean (± s.d.) amount (mg) found by the proposed method (n = 5)	Mean (± s.d.) % labelled amount (n = 5)
I	10	9.97 ± 0.03	99.96 ± 0.05
II	10	10.05 ± 0.23	100.5 ± 0.12

It can be concluded that the proposed HPLC method is sensitive, rapid and reproducible for the analysis of ezetamibe in bulk and tablet dosage forms.

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