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Simultaneous estimation of simvastatin and ezetimibe by RP-HPLC in pure and pharmaceutical dosage form

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ABSTRACT

A simple, precise and rapid RP-HPLC method was developed for the simultaneous determination of simvastatin and ezetimibe in combined pharmaceutical dosage forms. The method was carried out on a Shim-pack, RP-C18 column using a mixture of acetonitrile: methanol: buffer (triethylamine pH-3) in the ratio 15:45:40 and detection was done at 240 nm using external standard method as quantitation. The linearity range of simvastatin and ezetimibe were 0.5 to 20 µg/ml. The intra-day and inter-day precision were in the range of 1.02-1.43 and 0.53-0.94 for simvastatin, 0.24-1.29 and 0.93-1.32 for ezetimibe.

Key word: Simvastatin , Ezetimibe, Tablets, RP-HPLC.

INTRODUCTION

Simvastatin[1] (Fig 1), a hypolipidemic drug belonging to the class of statins is chemically designated as [(1S,3R,7R,8S,8aR)-8-[2-[(2R,4R)-4-hydroxy-6-oxo-oxan-2-yl]ethyl]-3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl]2,2-dimethylbutanoate. Various analytical methods have been reported for the determination of simvastatin, which include HPLC[2,3], HPLC-MS/MS[4], derivative spectrophotometry[5].

Ezetimibe[1] (Fig 1), a selective inhibitor of intestinal cholesterol and related phytosterol absorption, is designated as 1-(4-fluorophenyl)-3(R)-[3-(4-fluorophenyl)-3(S)-hydroxypropyl]-4(S)-(4-hydroxyphenyl)-2-azetidinone.Few HPLC methods for the determination of ezetimibe were reported in literature[6-7].

Although the combinational use of simvastatin and ezetimibe is continuously increasing, there are very few HPLC[8,9] methods for the simultaneous estimation of simvastatin and ezetimibe reported in literature. The availability of an HPLC method with high sensitivity and selectivity will be very useful for the simultaneous determination of simvastatin and ezetimibe in combined dosage forms. The developed method can be successfully applied to quality control and other analytical purposes.

EXPERIMENTAL

Reagents

Simvastatin (assigned purity, 99.7%) and ezetimibe (assigned purity, 99.9%) were gift samples from Ranbaxy labs, India. Acetonitrile and methanol of HPLC grade (E-Merck, India), triethyl

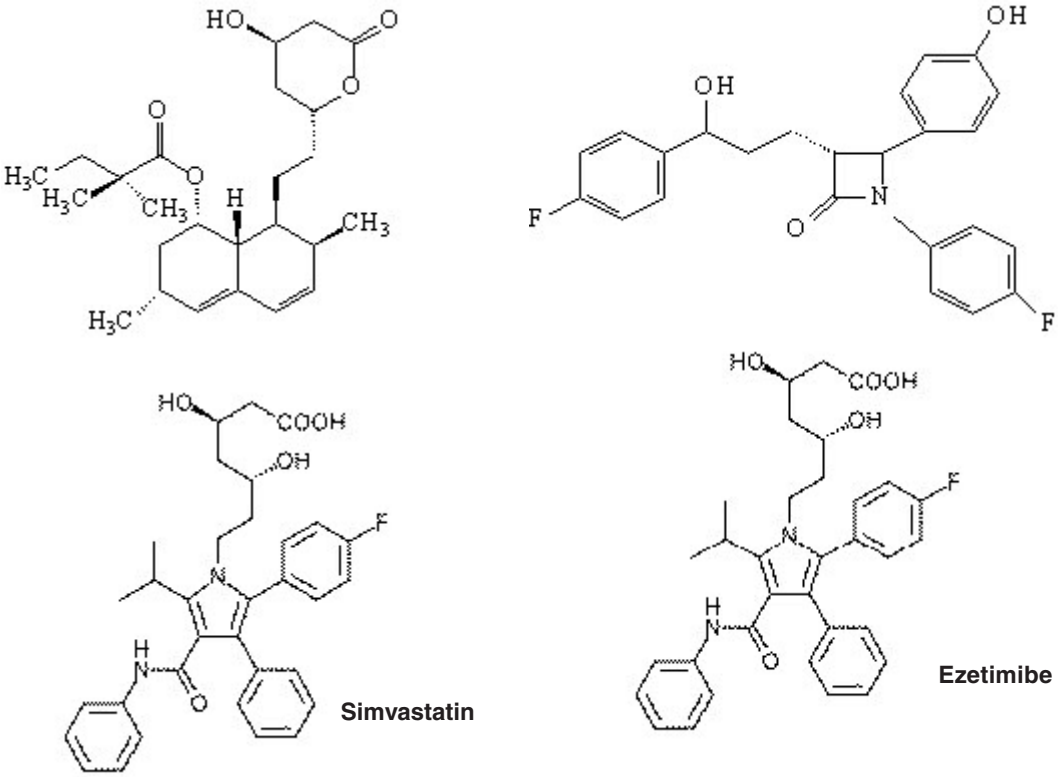

Fig 1: Chemical structures of Simvastatin and ezetimibe

amine, ortho phosphoric acid and HPLC grade water (Qualigen Chemicals, India) were used in the study. Commercially available these combined dosage forms claimed to contain 10 mg and 10mg of simvastatin and ezetimibe respectively and were procured from the local market.

Preparation of buffer

Add 7 ml of triethyl amine to 1 lit of HPLC water and adjust the pH to 3 with ortho phosphoric acid.

Apparatus

Quantitative HPLC was performed on a gradient high pressure liquid chromatograph (Shimadzu HPLC Class 10A series) with two LC-10AT pumps, a fixed wavelength programmable UV/VIS detector (SPD-10A), a guard column (Shimadzu TM, ODS 2 cm, Shim-pack) and RP C-18 column (250 mm × 4.6 mm i.d., particle size 5 µ) was used. The HPLC system was equipped with the software "Class LC-10AT series version 5.03" (Shimadzu).

HPLC conditions

The contents of mobile phase were mixture of acetonitrile: methanol: buffer (triethyl amine pH-3) in the ratio 15:45:40 v/v. They were filtered before use through a 0.45 µm membrane filter, degassed with a helium spurge for 15 min and pumped from the respective solvent reservoirs to the column at a flow rate of 1 ml/min, which yielded a column back pressure of 125-135 kg/cm^2. The run time was set at 12 min and the column temperature was maintained at 30°C. The volume of injection loop was 20 µl. Prior to injection of the drug solutions, the column was equilibrated for at least 30 min with the mobile phase flowing through the systems. The eluents were monitored at 240 nm and the data were acquired, stored and analyzed with the software Class LC-10AT series version Shimadzu.

Recommended procedure

A stock solution of the simvastatin and ezetimibe was prepared by dissolving 25 mg of simvastatin and 25 mg of ezetimibe in 25 ml of volumetric flask containing 15ml of methanol (HPLC grade) sonicated for about 15 min and then made up to volume with acetonitrile. Daily working standard solutions of simvastatin and ezetimibe were prepared by suitable dilution of stock solution with the diluent (methanol: acetonitrile 70:30). Five sets of the drug solution were prepared in the diluent, containing simvastatin and ezetimibe in the concentration of 0.5-20 µg/ ml. Each of these drug solutions (20 µl) was injected six times into the column and the peak area and retention times were recorded.

Procedure for pharmaceutical formulations

Not fewer than twenty tablets were weighed to obtain the average tablet weight and were then powdered. A sample of the powdered tablets, claimed to contain 25 mg of simvastatin and 25 mg of ezetimibe in a 25 ml volumetric flask. Then contents were dissolved in the volume that was made up with diluent. This mixture was shaken well and was then filtered through a 0.45 µm membrane filter. An aliquot of this solution (0.1 ml) was transferred to a volumetric flask and made up to a sufficient volume with diluent to get a concentration of 100 µg/ ml. From this, 1 µg/ ml of the solution was prepared. Various volumes of this aliquot were diluted to get test concentrations between 80-120% with diluent. All determinations were conducted in triplicate. The same procedure was used to estimate the concentration of the drug in two different strengths of tablets.

RESULTS AND DISCUSSION

The development of HPLC methods for the simultaneous determination of drugs has received considerable attention in recent years because of

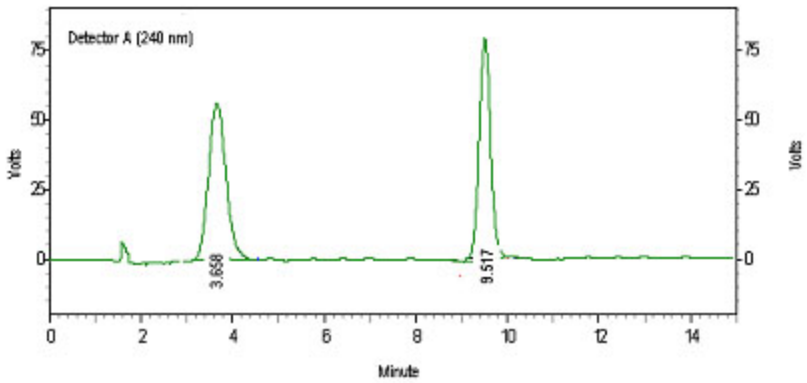

Fig. 2: A typical chromatogram of simvastatin and ezetimibe reference substance.

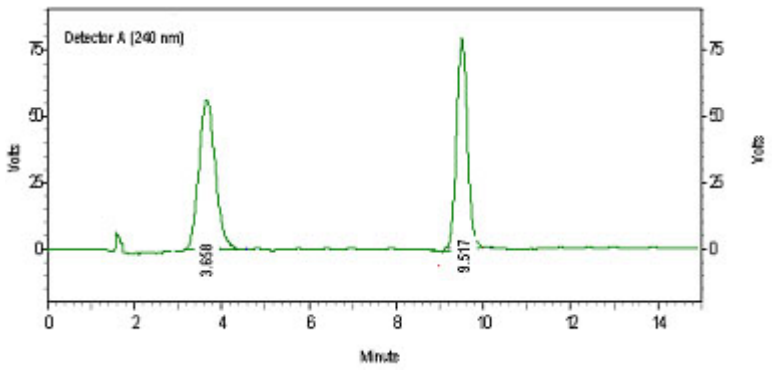

Fig. 3: A typical chromatogram of simvastatin and ezetimibe tablets

their importance in the quality control of drugs and drug products. The goal of this study was to develop a rapid HPLC method for the analysis of simvastatin and ezetimibe in its combined pharmaceutical dosage form, using the most commonly employed RP C-18 column with UV detection. The retention time of simvastatin and ezetimibe were found to be 3.65 and 9.51 min, respectively. Typical chromatograms of simvastatin and ezetimibe were shown in Fig. 2-3.

The calibration curve of simvastatin and ezetimibe was constructed by plotting the peak area of drug (Y) to the concentration (X). It was found to be linear with a correlation coefficient of 0.9999 and 0.9999, the representative linear regression equation being Y= 13694.42X-0.818 and Y= 11321.78X + 2.677. This method was validated for its intra and inter day precision. In the range of 0.5-20 µg/ ml, the relative standard deviations of five triplicate injections were found to be 0.53% -

Table 1: Calibration of the proposed HPLC method

Concentration (µg/ml)		Average area	
Simvastatin	**Ezetimibe**	**Simvastatin**	**Ezetimibe**
0.5	0.5	6847.25	5660.35
1	1	13698.32	11321.89
5	5	68460.94	56619.31
10	10	136945.81	113220.21
20	20	273885.54	226436.92

Table 2: Inter and Intra day precision for Simvastatin and ezetimibe assay in combined pharmaceutical dosage forms by proposed HPLC method

Concentration (µg/ml)		Observed concentration(µg/ml) Intra-day				Inter-day			
		Mean (n = 5)		% RSD		Mean (n = 5)		% RSD	
SMV	**EZM**	**SMV**	**EZM**	**SMV**	**EZM**	**SMV**	**EZM**	**SMV**	**EZM**
15	15	14.91	14.99	1.02	0.24	15.08	15.01	0.53	1.32
25	25	25.06	25.01	1.43	1.29	24.99	24.95	0.94	0.93

EZM - Ezetimiblel SMV - Simvasatin

Table 3: Mean (±S.D) amount of Simvastatin and Ezetimibe in tablet dosage forms by proposed HPLC method

Labeled amount		Observed amount		% purity	
Simvastatin	**Ezetimibe**	**Simvastatin**	**Ezetimibe**	**Simvastatin**	**Ezetimibe**
10	10	10.04±0.23	9.98±0.28	100.4±0.13	99.8±0.0.05
10	10	10.03±0.46	10.02±0.0.65	100.3±0.24	100.2±0.18

0.94% and 0.93% -1.32% respectively (Table 1). The inter assay precision (3 days, n = 6) was expressed as relative standard deviation and range between 0.53-0.94% and 0.93-1.32 % for simvastatin and ezetimibe respectively (Table 2).

The HPLC method developed in the present study was used to quantify simvastatin and ezetimibe in tablet dosage forms. The obtained results are given in Table 3. No interfering peaks were found in the chromatogram, indicating that the

Table 4: Recovery values of Simvastatin and Ezetimibe by proposed method

Concentration(µg/ml)		% Recovery	
Simvastatin	**Ezetimibe**	**Simvastatin**	**Ezetimibe**
80% sample 1	80% sample 1	100.25	99.61
80% sample 2	80% sample 2	101.34	100.74
80% sample 3	80% sample 3	99.41	100.25
100% sample 1	100% sample 1	100.41	100.26
100% sample 2	100% sample 2	99.63	101.38
100% sample 3	100% sample 3	99.68	101.00
120% sample 1	120% sample 1	99.88	99.01
120% sample 2	120% sample 2	100.03	99.75
120% sample 3	120% sample 3	99.82	99.98

tablet excipients did not interfere with the estimation of the drug by the proposed HPLC method. Also, when a known amount of the drug solution was added to a powdered sample of the tablet dosage form and subjected to an estimation of the drug by the proposed method, there was a high recovery (Table 4) of simvastatin and ezetimibe, indicating that the proposed procedure for the determination of simvastatin and ezetimibe in combined tablet dosage forms is highly accurate.

The results of the study showed that the proposed RP-HPLC method is simple, rapid, precise and accurate. It will be useful for the determination of simvastatin and ezetimibe in its combined pharmaceutical dosage forms.

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