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QUANTITATIVE ANALYSIS OF SIMVASTATIN AND EZETIMIBE OF DRUGS IN COMBINED DOSAGE FORMS BY HPLC

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ABSTRACT

A high performance liquid chromatographic method was developed for the quantitative analysis of drugs in a combined dosage form. The separation was effected on a phenomenex C_{18} column (250mm x 4.6mm i.d. particle size 5µ) using mobile phase consisting of 1M ammonium acetate buffer and acetonitrile (55: 45 v/v) at a flow rate 1.8mL/min. The detection was made at 230nm the retention times for ezetimibe and simvastatin were 4.5 and 20.1 min respectively. Calibration curves were linear over the ranges of 10-50µg/mL for ezetimibe and simvastatin. The proposed method was validated as per the ICH and USP guidelines. The method is accurate, precise and found to be suitable for the quantitative analysis of drugs in a combined dosage form.

KEYWORD

Simvastatin, Ezetimibe, HPLC

INTRODUCTION

Simvastatin (SIM) butanoic acid, 2, 2- dimethyl-, 1, 2, 3, 7, 8, 8a-hexahydro-3, 7-dimethyl-8-[2(tetrahydro-4-hydroxy- 6-oxo-2H-pyran-2-yl)-ethyl]-1-naphthalenyl ester, is a lipid-lowering agent that is derived synthetically from fermentation products of *Aspergillus terreus*[1]. After oral ingestion SIM, this is an inactive lactone, is hydrolyzed to corresponding ortho-hydroxy acid leading to the inhibition of 3-hydroxy 3-methyl glutaryl – coenzyme A. (HMG- CoA) reductase, responsible for catalyzing the conversion of HMG CoA to mevalonate[2], which is an early and rate limiting step in cholesterol biosynthesis. Ezetimibe (EZ), 1- (4-Fluorophenly) – 3 (R) - [3-(4-fluorophenyl) - 3 (S) hydroxy propyl]-4 (S)–(4-hydroxy phenyl) – 2 azetidinones, is a therapeutically beneficial drug that works by inhibiting the protein transporters on small intestinal brush border, which brings about this active transport of cholesterol. In addition, it also inhibits phytosterol absorption[3].Clinical studies have shown that co-administration of ezetimibe with statins could provide an additional reduction in LDL cholesterol as well as total cholesterol[4]. In addition, it also inhibits phytosterol absorption.[5] EZ has no inhibitory effect on absorption of lipid soluble

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vitamins triglycerides or bile acids, as do statins. This distinct mechanism of action results in a synergistic cholesterol lowering effect, when used together with statins that inhibits cholesterol synthesis by liver[6]. A few methods based on HPLC[7-11],UV [12,13], LC-MS[14,15] and GC-MS[16] was reported earlier for the determination of simvastatin individually and in combination with other drugs. A few analytical procedures were also proposed for the determination of ezetimibe in dosage forms[17] in human serum[18-20], urine and feces[21]. This paper now describes an HPLC method for the simultaneous determination of simvastatin and ezetimibe in tablets. The method is rapid, accurate and precise.

EXPERIMENTAL

Instrumentation

The present work was carried out on gradient high pressure liquid chromatograph (Shimadzu HPLC class VP series) with Shimadzu HPLC SPD-10 ATVP pump, variable wavelength programmable UV-Vis detector SPD-10AVP system and operating software winchrom was used. The chromatography column used was a reverse phase phenomenax C_{18} column (250mm x 4.6mm i.d.particle size 5µ), including Rheodyne valve injector with 20 µl fixed loop. A commercial sample of tablets of Simlo10 EZ of Lessac research laboratories, Mumbai, Simvas EZ 10 of Micro laboratories, Bangalore and Simcard EZ 10 of Cipla Pharmaceuticals, Mumbai containing SIM and EZ in ratio of 10 mg : 10 mg respectively were purchased from local market.

Reagents and Chemicals

SIM and EZ were obtained as gift samples from Micro labs, Bangalore, India. All solvents were of HPLC grade obtained from Merck Research Laboratory, Mumbai, India. HPLC grade water were purchased from Qualigens fine chemicals Mumbai, India.

Experimental conditions

The HPLC system was operated isocratically at flow rate of 1.8 ml/min at $25^{o}C \pm 0.5^{o}C$. Thermobile phase found to be most suitable for analysis was acetonitrile: water in the ratio of 55: 45 v/v of acetonitrile: buffer solution (1 gm of Ammonium acetate in 1000ml water).

Preparation of standard solution

Standard stock solution of 1000µg/ml of each SIM and EZ were prepared by dissolving 100 mg of each drug in acetonitrile and buffers solution. Sub stock solution was prepared from Stock solution by diluting each standard stock solution (10 ml) up to 100 ml to get 100 µg/ml of both drugs. The nominal concentrations in range of 10 to 50 µg/ml were prepared for calibration. Tablets of SIM and EZ combination are available in 1:1 ratio.

Sample Preparation

Twenty tablets were weighed and crushed to fine powder. Powder equivalent to 10 mg of SIM was weighed and dissolved to acetonitrile and buffer solution, sonicated for 10 min and filtered through whatmann filter paper No. 42; finally different concentrations of tablet sample were prepared by serial dilution technique.

PROCEDURE

Chromatographic conditions

Chromatographic separation was achieved by using mobile phase consisted of acetonitrile: water in the

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ratio of 55:45 %v/v, following through Phenomenex C_{18} column was constant flow rate of 1.8ml/min. A phenomenex C_{18} column was used as the separation phase. Detection was carried out using a UV detector at 230nm.

Linearity

To establish the linearity a series of dilutions ranging from 50-150 µg/ml for SIM and 50-150µg/ml for EZ were prepared separately and calibration graph was plotted between the mean peak area Vs respective concentration and regression equation was derived.

Y_{SIM} = 933.68x + 3360.8 (r2 = 0.9939)

Y_{EZ} = 758.52x + 3723.8 (r2 = 0.9915)

Method validation

The accuracy, precision and robustness were determined by analyzing a set of laboratory sample (n=3) with each of the five concentrations ranging from 10-50µg/ml for both drugs. Precision of analytical method was studied in terms of repeatability (intra-day) and inter mediate precision (inter-day). Repeatability was checked by analyzing three independent samples of both the drugs at 10 percent concentration levels and percent relative standard deviation (%RSD) was calculated. To determine the intermediate precision, mixed solutions of simvastatin and ezetimibe at three different concentration levels were analyzed three times with the same day (intra-day) and on three different days (inter-day variation). The results are shown in table 1.

TABLE 1.

INTER-DAY AND INTER DAY ASSAY OF THE PROPOSED METHOD

Concentration of drug.(µg/ml)	Precision data			
	Intra-day precision % RSD		Inter-day precision % RSD	
	Simvastatin	Ezetimibe	Simvastatin	Ezetimibe
10	0.32	0.08	0.53	0.60
20	0.59	0.27	0.41	0.82
30	0.68	0.61	0.91	0.58

RESULTS AND DISCUSSION

Chromatographic method

Initially methanol was tried as mobile phase in which SIM and EZ shows greater peak symmetry which was not satisfying the system suitability criterion and the resolution requirement for the simultaneous estimations of these two drugs. The tailing for both the peaks was reduced considerably by adding

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acetonitrile in the mobile phase and brought close to 1, which is ideal requirement for chromatographic analysis. Acetonitrile can help in reducing the viscosity of the mobile phase and hence reduce the backpressure and increase the column life. A representative graph of this is shown Figure.1.

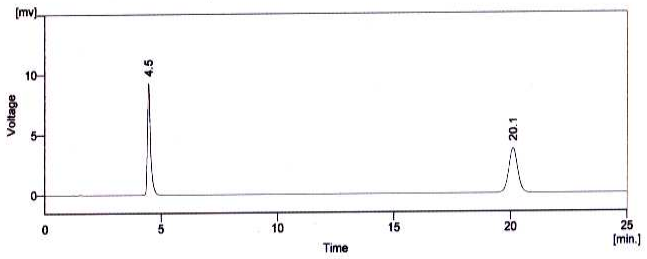

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Figure1.
Representative chromatogram of Simvastatin and Ezetimibe

System suitability

The system suitability test was applied to a representative chromatogram to check the various parameters such as column efficiency, resolution, precision and peak tailing. The result obtained is shown in Table 2. The retention time for SIM and EZ was 20.1 and 4.5 respectively. The number of theoretical plate for SIM and EZ were 13312 and 6659. All these parameters were evaluated with the background of regulatory requirements which also suggests good chromatographic conditions.

TABLE 2.
SYSTEM SUITABILITY PARAMETERS.

Parameters	Values found in Simvastatin	Values found in Ezetimibe
Tailing factor	1.12	2.08
RSD	0.0667	0.0564
No of theoretical plates	13312	6659
Resolution	34.23	-----
Calibration range	10-50µg/ml	10-50 µg/ml

Accuracy and Precision

The recovery experiment was carried out by spiking the already analyzed sample of the tablets with their different known concentration of standard SIM and EZ. The result is summarized in Table 3. The percent recovery for SIM ranges from 100.04-100.43 % and EZ ranges from 100.06-100.16 %.

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TABLE 3.
RECOVERY STUDIES DATA SHOWING AMOUNT OF DRUG RECOVERED FROM SAMPLE SOLUTION AND AVERAGE RECOVERY

Drug	Tablet amount (µg/ml)	Amount added (µg/ml)	Amount recovered (µg/ml)	Recovery (%)	Coefficient of variation
Simvastatin	10	50	50.21	100.06	
	10	75	75.11	100.04	
	10	100	100.15	100.29	0.999
	10	125	125.12	100.43	
	10	150	150.12	100.20	
	10	50	50.22	100.14	
Ezetimibe	10	75	75.20	100.16	0.999
	10	100	100.12	100.06	
	10	125	125.21	100.11	
	10	150	150.13	100.13	

Assay

The content of SIM and EZ found in the tablets by the proposed method are shown in Table 4. The low R.S.D indicates that the method is precise and accurate.

TABLE 4.
ANALYSIS OF DOSAGE FORMS

Samples	% Assay of Simvastatin	% Assay of Ezetimibe
Simvas EZ	99.4	99.7
Simlo 10 EZ	100.9	100.7
Simcard EZ	100.6	101.3
MEAN	100.3	100.56
% RSD	0.77	0.80

Stability sample solution
The sample solution injected after 12 hrs did not shows any appreciable changes.

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CONCLUSION

The proposed HPLC method allows for accurate, precise and reliable measurement of SIM and EZ simultaneously in combined dosage form. The developed HPLC method was found to be simple, rapid, selective, accurate and precise for the concurrent estimation of drugs in respective two-component tablet dosage from of SIM and EZ. The RSD for all parameters was found to be less than one. Which indicates the validity of method and assay results obtained by this method and assay results obtained by this method are in fair agreement. The developed method can be used for routine quantitative simultaneous estimation of SIM and EZ in multi-component pharmaceutical preparation.

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