



SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF SIMVASTATIN AND EZETIMIBE IN BULK DRUG AND ITS COMBINED DOSAGE FORM.

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ABSTRACT

A simple, accurate, precise, sensitive and a highly selective spectrophotometric method was developed for the simultaneous estimation of simvastatin and ezetimibe. The estimation of simvastatin was carried out by dual wavelength method at 223 nm and 254.5 nm while ezetimibe was estimated as single component at 258.5 nm. The method was found to be linear in the range of 1 – 25 µg/ml with mean recovery of 99.0 % and 99.65 % of simvastatin and ezetimibe, respectively. The developed method was validated according to ICH guidelines and it found to be accurate and precise Thus the proposed method can be successfully applied for simultaneous determination of simvastatin and ezetimibe in routine analysis work.

Keywords: Simvastatin, Ezetimibe, Spectrophotometric.

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, which is an inactive lactone, is hydrolyzed to corresponding β-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, which is an early and rate limiting step in cholesterol biosynthesis².

Ezetimibe (EZ), 1- (4-Fluorophenyl) – 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 absorption³. EZ has no inhibitory effect on absorption of lipid soluble 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 liver⁴.

Recently, a combination of SIM and EZ has been launched in the market. In this combination, EZ shows a synergistic effect with SIM.

SIM may be determined by several methods including gas chromatography–mass spectrometry (GC–MS)⁵, liquid chromatography with UV detection (LC–UV)⁶⁻⁸. EZ was determined with or without combination of several drug by HPLC and spectrophotometrically^{9,10}. Literature survey revealed that there is no UV method has been reported yet for the analysis of these two drugs in combination without preliminary separation that makes it worthwhile to pursue the present work.

EXPERIMENTAL

Instrumentation

The present work was carried out on Shimadzu UV- 1700 series spectrophotometer having double beam detector configuration. The absorption spectra of reference and test solution were carried out in a 1 cm quartz cell over the range of 220-320 nm.

Reagent and chemicals

SIM and EZ were obtained as gift sample from Ranbaxy Lab, Dewas,

India. All solvents were of HPLC grade obtained from Merck Research Laboratory, Mumbai, India.

Experimental condition

According to the solubility characteristics of drug a combination of methanol and phosphate buffer (7.4 pH) in 7:3 ratio was selected as solvent for analysis. From the overlay spectra two wavelengths were selected for estimation of SIM (223 nm and 254.5 nm) where EZ shows similar absorbance and the estimation of EZ on one wavelength (258.5 nm) was performed where SIM has no significant absorbance, so EZ can be estimated as single component

Standard stock and sub stock solution

UV analysis was done by using the standard stock solution of 1000 µg/ml of each SIM and EZ by dissolving 100mg of each standard drug separately in mixture of methanol and phosphate buffer (7.4 pH) in 7:3 ratio. Aliquots of 5, 10, 15, 20, 25 µg/ml were prepared by using this stock solution, for the preparation of calibration curve.

Working standard solution

Tablets of SIM and EZ combination are available in 1:1, 2:1, 4:1 & 8:1 ratio. Working standards were prepared in the ratio of 2: 1 from standard stock solution of 1000 µg/ml.

Sample preparation

Twenty tablets were weighed and crushed to fine powder. Powder equivalent to 20 mg of SIM was weighed and dissolved in mixture of methanol and phosphate buffer (7.4 pH) in ratio of 7:3, 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

Spectral characteristics of SIM and EZ

Aliquots of sub stock solution equivalent to 200 µg and 100 µg of SIM and EZ respectively, were transferred separately into 10 ml volumetric flask and the volume was made with diluent. The absorption spectra of both the drugs were recorded from 200-400 nm.

Wavelength selection

For Estimation of SIM, two wavelength 223, 254.5 nm were selected and calibration curve (n = 6) was plotted in the range of 5 - 25µg/ml between absorbance and concentration. EZ was estimated as single component at 258.5 nm where SIM shows no interference. Calibration curve was plotted between absorbance and its nominal concentration. Calibration curve equation was used to calculate the concentration of SIM and EZ in laboratory samples.

Linearity

The linearity for spectrophotometric method was established in the concentration of 5, 10, 15, 20 and 25µg/ml for both the drugs.

Method validation

Accuracy was determined by recovery study. 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. Precision for assay were determined by repeatability, inter day, intra day precision for both drugs (each in three replicate).

RESULT AND DISCUSSIONS

Spectral characteristics of SIM and EZ revealed that there was a considerable spectral overlap in the range of 200 - 400nm. It was observed that at the point of maximum absorbance of SIM there is interference of EZ but at 258.5nm of EZ spectra, there is no interference of SIM. Therefore, SIM was estimated at two wavelengths where EZ has similar absorbance and EZ can be estimated as single component at 258.5 nm as there is no interference of SIM. (Fig.1).

Linearity

For estimation of SIM, calibration curve (n = 6) was plotted in the range of 5–25 µg/ml between absorbance difference at 223, 254.5 nm and concentration of SIM

and for EZ, absorbance of EZ and its concentration at 258.5 nm. Calibration curve equation was found to be $ABS_{SIM} = 0.0364 C + 0.0018$ for SIM and $ABS_{EZ} = 0.0265 C + 0.0048$ for EZ. The correlation coefficient was found to be (r^2) 0.9999 and 0.9996 for SIM and EZ, respectively

Assay

The amount of SIM and EZ found in

the tablets by the proposed method are shown in Table 1. The low value of R.S.D. indicates that the method is precise and accurate.

Accuracy and precision

The result of accuracy is summarized in Table 2.

The recovery for SIM ranges from 98.07 to 99.48 % and EZ ranges from 99.19 to 99.93 %.

Table 1: Result of statistical analysis of the tablet dosage form.

	SIM	EZ
Simvastatin EZ 20 % Mean	100.03	99.696
S.D*	0.2278	0.5604
%COV**	0.23	0.57

* Standard deviation **Percent Coefficient of variation.

Table No.2- Recovery studies data showing amount of drug recovered from sample solution and average recovery

	Amount present ($\mu\text{g/ml}$)	Amount found ($\mu\text{g/ml}$)	% Recovery
SIM			
1	25.11	24.98	99.48
2	30.13	29.55	98.07
3	35.21	35.02	99.46
Average			99.00
S.D			0.80
EZ			
1	15.09	15.08	99.93
2	20.11	20.08	99.85
3	24.86	24.65	99.19
Average			99.65
S.D			0.40

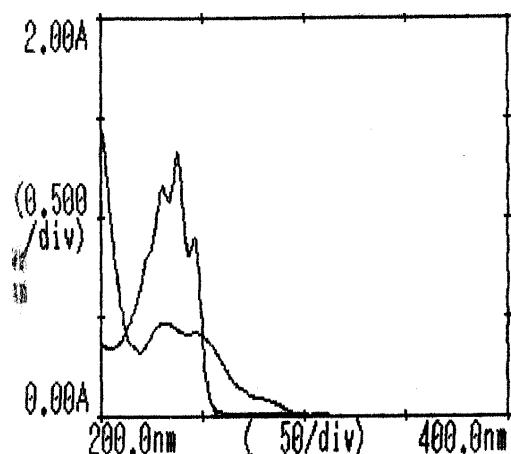


Fig. 1 : Overlay spectra between sim and Ez

Overlay spectra of SIM and EZ shows that, at the wavelength of 223 and 254.5 nm of SIM spectra, EZ shows similar absorbance where as at 258.5 nm of EZ spectra SIM not shows any absorbance.

CONCLUSIONS

The proposed spectrophotometric method is accurate, precise and reliable for the simultaneous measurement of SIM and EZ in combined dosage form. The developed spectrophotometric method was validated for simultaneous estimation of SIM and EZ using linearity, range, accuracy and precision. 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 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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