DEVELOPMENT AND VALIDATION OF SPECTROPHOTOMETERIC METHOD FOR SIMULTANEOUS DETERMINATION OF SIMVASTATIN AND EZETIMIBE IN TABLET FORMULATIONS

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ABSTRACT

A versatile, accurate, precise and economic method for simultaneous determination of simvastatin and ezetimibe in fixed dose combination products was developed. The absorbance values at 236 nm and 234 nm of over line spectrum was used for the estimation of simvastatin and ezetimibe, respectively without mutual interference. This method obeyed Beer's law in the concentration range of 4 – 16 µg /ml for simvastatin and 4-16 µg /ml for ezetimibe. The results of analyses have been validated statistically for linearity, accuracy and precision of the proposed method.

Keywords: Simvastatin (SMV), Ezetimibe (EZE), methanol, ultraviolet spectrophotometry, absorption ratio method.

INTRODUCTION

Simvastatin (SMV) is an oral antilipidaemic agent, chemically it is a butanoic acid derivative, 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. Simvastatin was analysed by HPLC (Carlucci G *et al.,* 1992 a, b) and LC-MS (Zhang NY *et al.,* 2004) and second derivative by UV spectrophotometry (Wang L, and Asgharnejad M. 2000) methods in pharmaceutical preparations when present alone. Ezetimibe (EZE) is also an oral antilipidaemic agent and is chemically 1-(4-fluorophenyl)-(3R)-[3-(4 fluorophenyl)- 3S)-hydroxy-phenyl]-4S-(4-hydroxyphenyl)-2-azetidinone. It is not official in any pharmacopoeia. HPLC (Sistla RM, *et al.,* 2005) and LC-MS (Kosoglu T *et al.,* 2004) methods have been reported for the estimation of EZE in pharmaceutical formulations and in plasma. Also, HPLC (Shivshankar K, *et al.,* 2007, Lingeswara Rao Punati *et al.,* 2006) methods were reported for the simultaneous estimation of SMV and EZE in combined dosage forms. The review of literature revealed that no method is reported for the simultaneous estimation of SMV and EZE in fixed dose combination products by UV spectrophotometry. The present research describes a simple, rapid, accurate and reproducible method for the simultaneous estimation of SMV and EZE in tablet formulation by Absorption ratio method.

EXPERIMENTAL WORK

Materials

Pharmaceutical grade of Simvastatin and Ezetimibe were kindly supplied as gift sample by Dr. Reddy's Laboratory. Four different formulations of SMV and EZE namely Vytorin (Merck/Schering-Plough Pharmaceuticals (MSP)), Inegy (Bayer Pharmaceuticals), Zintrepid (SkyePharma) and Starstat (Lupin Laboratories) were obtained from retail pharmacies. Each containing 10 mg EZE and 20 mg SMV. Methanol AR Grade was procured from Qualigens Fine chemicals, Mumbai.

Equipments

Single beam systronics U.V. visible spectrophotometer 117 with two matched cuvette cells of one cm light path were used for the measurement of absorbance. Electronic Dhona Balance 200D was used for weighing the samples. Class 'A' volumetric glassware were used.

PROCEDURE

Development of the method

The solutions of SMV and EZE were prepared separately in methanol at a concentration of 6 µg /ml. They were scanned in the wavelength range of 200-400nm. Data were recorded at an interval of 1nm. Then the spectra of the two drugs were drawn to obtain the overline spectra. The wavelength selected for SMV analysis was 236nm and for EZE analysis was 234nm as maximum wavelength respectively. The overlain spectra of the drugs are shown in fig. 1.

Construction of calibration curve

Standard stock solutions were prepared by dissolving 100mg of each standard drug samples in 100ml volumetric flask separately and the volume was made up with methanol to get a concentration of 1mg/ml. From this, suitable dilutions were made in methanol to get the working standard solutions of 4-16µg /ml for SMV and 4-16µg /ml for EZE separately. The absorbances of the

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spectra were measured at 236nm and 234nm for SMV and EZE, respectively. Six replicate analyses were carried out. Absorbance vs concentrations were plotted to obtain the calibration graph. Both the drugs obeyed Beer's law with the above Concentration range with 'r' value of 0.9996 and 0.9998 for SMV and EZE, respectively (figs. 2 and 3). Table 1 shows regression analysis data.

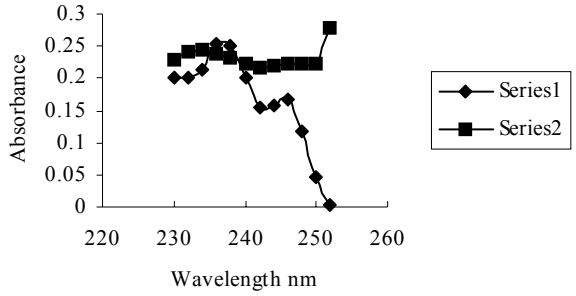

Fig. 1: Over line spectra of Ezetimibe and Simvastatin
Note: Series 1 =EZE, Series 2=SMV

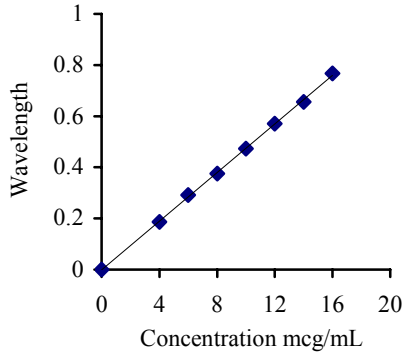

Fig. 2: Calibration curve of Simvastatin

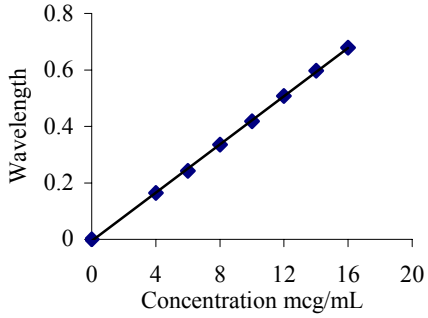

Fig. 3: Calibration curve of Ezetimibe.

The absorptivity values at 236nm and 234nm for both drugs were determined by checking absorbance values over a concentration range 4-16mcg/mL for working standards (table 2).

Method (Absorption ration / Q Method analysis)

From the over line spectrum of SMV and EZE, one wavelength was selected for the estimation of both drugs, which is known as isoabsorptive point (at 239nm). The dilutions of standard and sample solutions were prepared. The absorptivity values were determined at 239nm. The method employs Q values and the concentrations of drugs in sample solution were determined by using the following formula:

For simvastatin:

$$C_1 = \frac{Q_m - Q_y}{Q_x - Q_y} \times \frac{A}{a_x} \quad (1)$$

For ezetimibe:

$$C_2 \frac{Q_m - Q_x}{Q_y - Q_x} \times \frac{A}{a_y} \quad (2)$$

Where,

A = absorbance of sample at its isoabsorptivity point.

$$Qo = \frac{\text{Absorbance of sample at 234nm}}{\text{Absorbance of sample at 239nm}}$$

$$Q_{EZE} = \frac{\text{Absorptivity of Ezetimibe at 234nm}}{\text{Absorptivity of Ezetimibe at 239nm}}$$

$$Q_{SMV} = \frac{\text{Absorptivity of Simvastatin at 234nm}}{\text{Absorptivity of Simvastatin at 239nm}}$$

a_x & a_y are absorptivities of Ezetimibe and Simvastatin respectively at isoabsorptivity point. The results of the analysis of marketed formulation were reported in table 3.

Procedure for analysis of tablet formulation

Twenty tablets of each formulation were powdered. The powder equivalent to 10 mg of SMV and EZE was weighed accurately and transferred to 100 ml volumetric flask. Twenty ml of methanol was added to the flash and sonicated for 20 min. the solution was filtered through Whatman filter paper No. 41 and the volume was adjusted to the mark with methanol. This solution is expected to contain 100 µg/ml SMV and 100 µg/ml EZE. From the stock solution 1 ml was taken into a 10 ml volumetric flask and the volume make up to the mark with methanol to get a final concentration of SMV (10 µg/ml) and EZE (10 µg/ml). Absorbances of the sample solution were recorded at 239 nm & 234 nm. The concentration of two

drugs in sample was determined by using the equations (1) and (2).

The amount of drug present in the given tablet was determined by using the formulae 3 & 4.

$$\text{Amount of SMV present in given tablet} = \frac{C1 \times \text{dilution factor} \times \text{avg.weight}}{\text{Weight taken}} \quad (3)$$

$$\text{Amount of EZE present in given tablet} = \frac{C2 \times \text{dilutionfactor} \times \text{avg.weight}}{\text{Weight taken}} \quad (4)$$

VALIDATION

Validation of the developed method was done according to the USP 2006, Asian edition (table 4).

Linearity

The linearity of the method is its ability to elicit test results that are directly proportional to the concentration of the analyte in samples. The calibration curve was taken in the range of 4-14 mcg/mL at the respective $\lambda_{max.}$ The correlation coefficient of the linearity was found to be 0.999 at each wavelength for both drugs as shown in table 1.

Precision

The precision of an analytical method is determined by assaying a sufficient number of aliquots of a homogeneous sample to be able to calculate statistically valid estimate of % Relative Standard Deviation (%RSD). Intermediate precision was done to express within laboratory variation, on different days. Five replicates of 12 µg/mL concentration of the working standard mixture and sample solution were analysed %RSD was found to be less than 2%.

Specificity

Results of tablet solution showed that there is no interference of the excipients when compared with the working standard solution. Thus, the method was said to be specific.

RESULT AND DISCUSSION

The proposed methods for simultaneous estimation of Simvastatin and Ezetimibe in combined tablet dosage form were found to be simple accurate economical and rapid. The % RSD was found to be less than 2% in the developed method. Hence proposed method may be used for routine analysis of these drugs in combined dosage forms.

Table 1: Regression analysis of calibration curves

Concentration mcg/mL	Absorbance of SMV at 236 nm	Absorbance of EZE at 234 nm	Absorbance at Isoabsorptive point (239 nm)
4	0.187	0.164	0.144
6	0.291	0.243	0.216
8	0.376	0.335	0.288
10	0.473	0.418	0.366
12	0.571	0.508	0.434
14	0.656	0.597	0.509
16	0.767	0.679	0.567
Correlation coefficient (r) Intercept Slope	0.9996 -0.00146 0.0475	0.9998 -0.0126 0.04332	0.9996 0.00246 0.071

Table 2: Absorptivity values for simvastatin and ezetimibe

Concentration mcg/mL	Absorptivity at 236 nm SMV	Absorptivity at 234 nm EZE	Absorptivity at Isoabsorptive point (239 nm)	
			SMV	EZE
4	46.3	41	36	36
6	46.8	40.5	36.3	36.3
8	47	41.8	36	36
10	47.3	41.8	36.6	36.6
12	47.5	42.38	36.1	36.1
14	46.8	42.64	36.3	36.3
16	47.9	42.4	35.4	35.4
Mean Standard deviation	47.08 0.527	41.7 0.782	36.1 0.374	36.1 0.374

Table 3: Analysis of marketed formulations

Formulation	Label Claim (mg/tab)		Amount Found (mg/tab)		% Label Claim	
	EZE	SMV	EZE	SMV	EZE	SMV
1	10	20	9.91	19.82	99.1	99.1
2	10	20	10.09	20.19	100.9	100.95
3	10	20	10.08	19.63	100.8	99.15
4	10	20	9.92	20.07	99.2	100.85

Table 4: Validation parameters

S. No.	Parameter	SMV	EZE
1	Linearity	0.9996	0.9998
2	Precision(RSD)	0.278	1.46
3	Regression equation	Y=-0.00146+0.0475x	Y=-0.0126+0.04332x
4	Beer's law limit	4 - 16mcg/mL	4 - 16mcg/mL

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