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Simultaneous Estimation of Simvastatin and Ezetimibe by UV Spectroscopy

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ABSTRACT: *Simvastatin (SMV) and Ezetimibe (EZT) are the antihyperlipidemic drug belonging to HMG-CoA reductase inhibitor and helps to reduce harmful cholesterol levels in blood. A rapid, specific and economic UV spectrophotometric method has been developed using Methanol and distilled water in the ratio of (40:60) as a solvent for simultaneous determination of Simvastatin and Ezetimibe content in bulk and pharmaceutical dosage formulations. The absorbance values at 246 nm and 229 nm were used for the estimation of Ezetimibe and Simvastatin. The absorption maxima of Ezetimibe and simvastatin shown at 246 nm and 229 nm and methanol and distilled water in the ratio of (40:60) was used as solvent. This method obeyed Beer's law in the concentration range of 3–18 µg /ml for simvastatin and 5-30 µg /ml for Ezetimibe. The Simultaneous Estimation method was developed and validated according to ICH guidelines for linearity, precision, accuracy, LOD and LOQ. Simvastatin found to be linear within concentration range of 3-18 µg/ml with regression coefficient of 0.998 and Ezetimibe found to be linear within concentration range of 5-30 µg/ml with regression coefficient of 0.999. The percentage RSD value for the SMV at the concentration of 3, 6 and 9 µg/ml and their average % RSD value were 0.350, 0.168 and 0.286 while for the EZT the concentration of 5, 10 and 15 µg/ml and their average % RSD value were 0.387, 0.170 and 0.282. The average % recoveries for three different concentrations was found to be 95.50 % for SMV and 96.60 % for EZT. The limit of detection and limit of quantification were found to be 0.33 µg/ml and 1.33 µg/ml for Simvastatin and 1.007 µg/ml and 4.04 µg/ml for Ezetimibe respectively by proposed UV spectrophotometric method. The results of validation parameters indicates that the developed method was also found to be accurate, precise and sensitive and such simple & economic method can be used for the simultaneous estimation of Simvastatin and Ezetimibe. The obtained results proved that the method can be employed for the routine analysis of simvastatin and Ezetimibe in bulks as well as in the commercial formulation.*

Keywords: *Simvastatin, Ezetimibe, Methanol, Distilled water, Simultaneous Estimation, UV spectrophotometry, ICH guidelines.*

I. INTRODUCTION

Most of the pharmaceutical industries, are manufacturing multiple drug formulation to meet the market demand. It is a well-known fact that a combination of drug has a wider range to treat ailment as compared to a single drug components. There are many method reported for simultaneous analysis of drug component of multiple component formulation. Almost all pharmacopoeial methods available for the analysis of such formulation are applicable only after prior separation of drug components. Hence making them tedious and time consuming. There is likely to be loss of accuracy and precision



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due to extraction or separation. In the pharmaceutical field, for assurance of the quality of drug formulation, it become necessary to develop analytical method which should have accuracy and precision. The accuracy and precision depend upon the relative and absolute errors. Errors will be less, if the method is simple. The method can be directly related to accuracy and precision. Therefore simplicity of method should be one of the prime consideration while developing the method of analysis. The instrument technique that can be utilize for analysis is UV-Visible spectrometry^{1,2}.

Simvastatin (SMV) and Ezetimibe (EZT) are the antihyperlipidemic drug belonging to HMG-CoA reductase inhibitor and helps to reduce harmful cholesterol levels in blood³.

The main objective of the study is to develop a rapid, specific and economic UV spectrophotometric method by using Methanol and distilled water in the ratio of (40:60) as a solvent for simultaneous determination of Simvastatin and Ezetimibe content in bulk and pharmaceutical dosage formulations⁴.

II. MATERIALS AND METHOD

MATERIALS

The drug samples, ezetimibe and simvastatin working standards were obtained as gift sample by Lupin Pvt. Ltd, Indore (MP) India. Starstat EZ 10 mg marketed tablets manufactured by Lupin pvt. Ltd. and Simvotin 10 mg manufactured by sun pharmaceutical limited was procured from local market. Methanol, and water used were analytical grade and were purchased from Merck Specialties Private Limited, Mumbai, India.

INSTRUMENTATION:

Variable wavelength programmable UV detector UV1800 double beam UV-Visible spectrophotometer was used to carry out spectral analysis and the data was recorded by Hitachi software. Sonicator (1.5L), Ultrasonicator was used to sonicating the mobile phase and samples. Standard and sample drugs were weighed by using Denver electronic chemical balance (SI-234) and pH of the mobile phase was adjusted by using Systronics digital pH meter.



III. EXPERIEMENTALS

3.1 PREPARATION OF STANDARED STOCK SOLUTION:

A. Standard Simvastatine stock solution (100 µg/mL)

Simvastatine standard stock solution was prepared by weighing 10 mg of Simvastatine and transferred to a 100 ml volumetric flask and volume was made up to 100 ml with Methanol & Water in the ratio of 40: 60 (Methanol: Water) to get a concentration of 100µg/ml, The prepared solution is sonicated for 5 minutes and filtered through the whatman filter⁵.

B. Standard Ezetimibe stock solution (100 µg/mL)

Ezetimibe standard solution was prepared by weighing 10 mg of EZT to a ten ml volumetric flask and volume was made up to 100 ml with with Methanol & Water in the ratio of 40: 60(Methanol: Water) to urge a degree of 100 µg/ml. The prepared solution is sonicated for 5 minutes and filtered through the whatman filter paper⁵.

C. Sample drug stock solution (100 µg/mL)

The sample drug solution was prepared by weighing 10 mg of powdred drug sample to a ten ml volumetric flask and volume was made up to 100 ml with with Methanol & Water in the ratio of 40: 60(Methanol: Water) to urge a degree of 100 µg/ml. The prepared solution is sonicated for 5 minutes and filtered through the whatman filter paper⁵.

3.2. DETECTION OF WAVELENGTH

The spectrum of diluted solutions of the ezetimibe and simvastatin in methanol was recorded using Shimadzu Spectrophotometer UV-1800 (Shimadzu Corp. Japan). The peak of Ezitimibe and Simvastatin was obtained at 246 nm and 229 nm. Which shows that drug is pure as given in the refrence⁶. The UV spectrum of Ezitimibe and Simvastatin are shown in the fig. 1 and 2.



Fig.1. UV Spectra of Ezetimibe

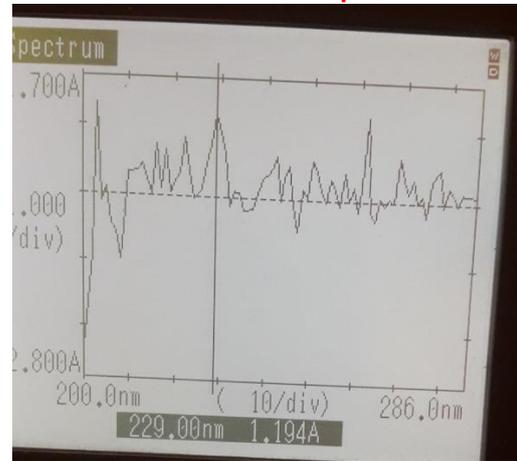


Fig.2. UV Spectra of Simvastatin

3.3 CALIBRATION CURVE

Calibration curve of Ezetimibe and Simvastatin was prepared in concentration range of 3-18 µg/mL Simvastatin 5-30µg/ml Ezetimibe with Methanol and Water in the proportion of 40:60⁷. The absorbance of each solution was measured at the wavelengths 246 nm and 229 nm. The absorbance values (mean of five determinations) with their standard deviation at different concentration in the range of 5-30 µg/ml and 3-18 µg/ml are shown in the fig. 3 and 4.

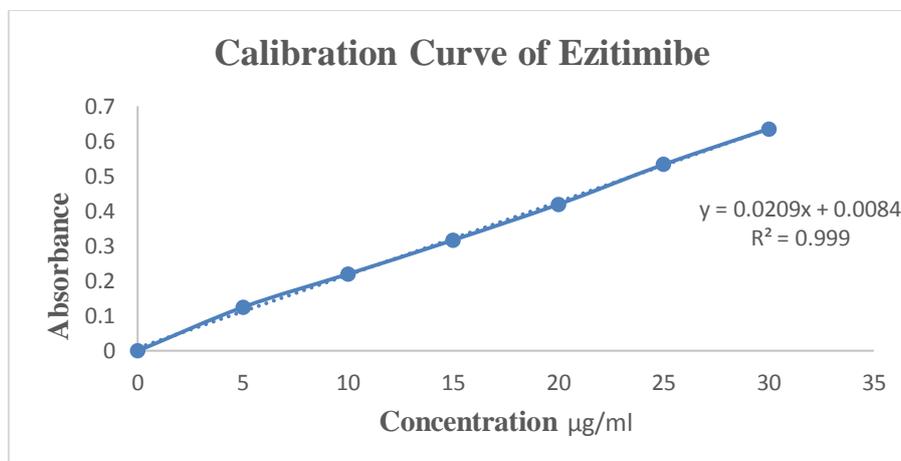


Fig. 3. Calibration Curve of Ezetimibe

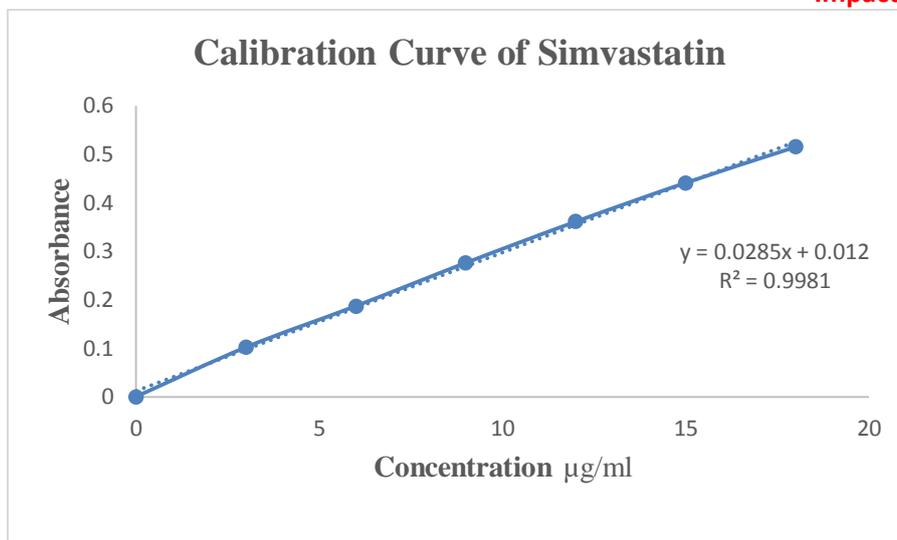


Fig.4. Calibration Curve of Simvastatin

IV. METHOD DEVELOPEMENT

SIMULTANEOUS ESTIMATION METHOD

The spectra of Ezetimibe and Simvastatine was used and wavelength 246 and 229 nm (λ max of EZT and λ max of SMV) were selected for the formation of the simultaneous equations. For calibration curves, stock solutions of Simvastatine and Ezetimibe within the concentration of range of 3 – 18 $\mu\text{g/ml}$ and 5 – 30 $\mu\text{g/ml}$ respectively. The absorbance of Simvastatine and Ezetimibe were measured at 229 and 246 nm, calibration curves were plotted. The absorptivities of both the drugs at both the wavelengths were determined.

The absorbance and the absorptivity values at the particular wavelength were calculated and substituted in the following equation, to obtain the concentration⁸.

$$\text{CSIM} = (A1ax2 - A2ax1) / (ax2ay1 - ax1ay2). \text{CEZB} = (A2ay1 - A1ay2) / (ax2ay1 - ax1ay2).$$

Where, CSIM = Concentration of Simvastatine CEZB = Concentration of Ezetimibe respectively,

A 1 = absorbance of sample at 248 nm, A 2 = absorbance of sample at 229 nm,



a_{x1} = absorptivity of Simvastatine at 229 nm and a_{x2} = absorptivityof Simvastatine at 246 nm, a_{y1} = absorptivity of Ezetimibe at 229 nm and a_{y2} =absorptivity of ezitimibe at 246 nm.

At $\lambda_{246\text{nm}}$: $1.9 = 0.19C_x + 0.19C_y$ (1)

At $\lambda_{229\text{nm}}$: $1.5 = 0.18C_x + 0.12C_y$ (2)

V. VALIDATION

Validation of the developed method was done according to the USP 2006, Asian edition.

5.1 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 3-18 $\mu\text{g/ml}$ for Simvastatine and 5-30 $\mu\text{g/mL}$ for Ezetimibe at the respective λ_{max} ⁹. The correlation coefficient of the linearity was found to be 0.999 at each wavelength for both drugs as shown in table 1.

Table 1: Regression analysis of calibration graphs of Simvastatin and Ezitimibe for proposed UV Spectrophotometric method

Sr. No.	Parameters	Results (Simvastatin)	Results(Ezitimibe)
1.	Absorption maxima	229 nm	246 nm
2.	Beer's range	3-18 $\mu\text{g/ml}$	5-30 $\mu\text{g/ml}$
3.	Regression equation	$y = 0.0285x + 0.012$	$y = 0.0209x + 0.0084$
4.	Correlation coefficient	0.9981	0.999
5.	Slope	0.0283	0.0211
6.	Intercept	0.012	0.0084

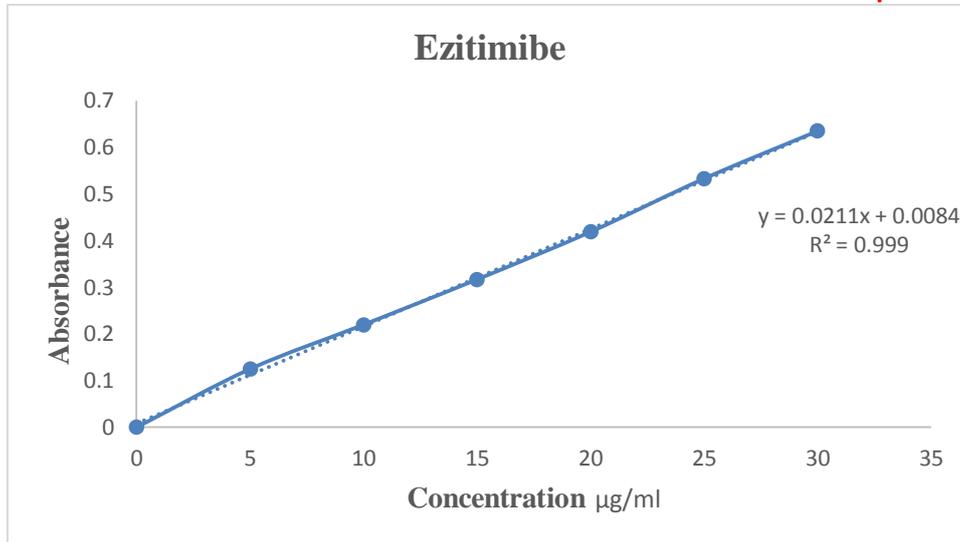


Fig. 5. Regression (Linearity) analysis for Ezitimibe

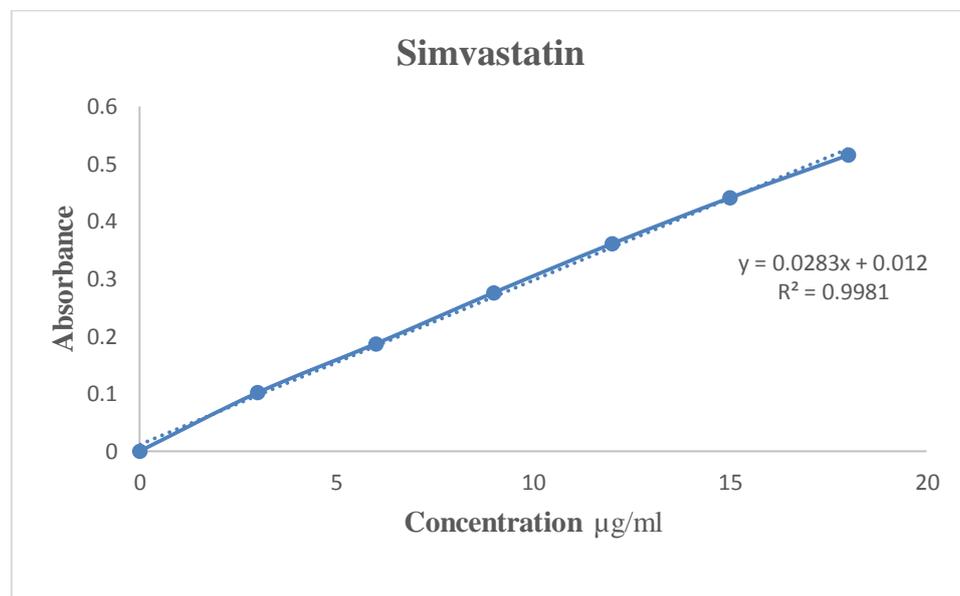


Fig. 6. Regression (Linearity) analysis for simvastatin

5.2 ACCURACY

In order to make sure the reliability and suitability of the proposed method, recovery studies were administered. It was done by mixing known quantity of ordinary drug with formulation sample and therefore the content were reanalysed by the proposed method. To a quantity of formulation like 10 mg of Simvastatin and Ezetimibe were added at 80%, 100% and 120% levels. This was extracted

diluted and reanalysed as per the formulation procedure. Absorbance were noted at respective wavelength¹⁰. Recovery studies were repeated for six times and the results are shown in table 2.

Table 2. Recovery (Accuracy) analysis for Simvastatin and Ezetimibe

S.no	Recovery Level	%Recovery	S.D %	% RSD	%Recovery	S.D %	% RSD
		Simvastatine			Ezetimibe		
1.	80 %	95.50	0.05670	0.2872	96.60	0.02732	0.2793
2.	100%	94.20	0.0258	0.3120	95.66	0.0429	0.5145
3.	120%	94.36	0.03240	0.70590	96.36	0.05123	0.6176

5.3 PRECISION

The precision of an analytical method is decided by assaying a sufficient number of aliquots of a homogeneous sample to be ready to calculate statistically valid estimate of twenty-two Relative Standard Deviation (%RSD). Intermediate precision was done to precise within laboratory variation, on different days. Five replicates of 8 µg/mL concentration of the working standard mixture and sample solution were analysed %RSD was found to be less than 2%¹¹. The results are shown in table 3.

Table 3. Result of Intraday (Repeatability) Precision studies

S.no	Parameters	% Amt. found (Simvastatin)			% Amt. found (Ezetimibe)		
		3 µg/ml	6 µg/ml	9µg/ml	5 µg/ml	10 µg/ml	15 µg/ml
1.	Morning	99.89	99.65	99.48	99.93	99.56	99.35
2.	Afternoon	99.69	99.69	100.05	99.66	99.97	99.98
3.	Evening	99.21	99.21	99.72	99.28	99.22	99.78
4.	Mean	99.59	99.79	99.75	99.48	99.84	99.76
5.	S.D.	0.349	0.168	0.286	0.385	0.170	0.282
6.	% R.S.D.	0.350	0.168	0.286	0.387	0.170	0.282

5.4 LIMIT OF DETECTION AND LIMIT OF QUANTIFICATION

It is the lowest amount of analyte and lowest concentration of analyte in a sample that can be detected but not necessarily quantitated under the stated experimental conditions. Limit of detection can be calculated using following equation as per ICH guidelines¹². The results are shown in table 4.

$LOD = 3.3 \times N/S$ Where,

N = Standard deviation of the response and S = Slope of the corresponding calibration curve

The limit of detection and limit of quantification were found to be 0.33 µg/ml and 1.33 µg/ml for Simvastatin and 1.007 µg/ml and 4.04 µg/ml for Ezetimibe respectively by proposed UV spectrophotometric method. Results of LOD and LOQ are summarized in (Table 4).

Table 4. Limit of detection (LOD) and limit of quantification (LOQ) of Simvastatin and Ezetimibe

S.no	Parameters	Method(Simultaneous estimation method)	
		Simvastatine	Ezetimibe
1.	LOD(µg/ml)	0.33068	1.3360
2.	LOQ(µg/ml)	1.0073	4.0485

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CONCLUSION

It can be concluded that the Simultaneous estimation and Method development for both the drugs was performed and it gave good results. The best result was given by methanol solvent.



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As method development procedure, validation studies were also performed for the same, but due to limited quantity of the compound only few parameters were observed as Linearity, limit of detection, Limit of quantification, Precision and linearity.

The validation procedure followed were as per the ICH guidelines. Simvastatin and Ezetimibe both gave excellent results. Since there were no reference results for this study, so the results were not compared to any standard.

The linearity was achieved with methanol solvent, Linearity, Accuracy and precision were satisfactory and the limit of detection (LOD), limit of quantitation achieved was also satisfactory. Hence we conclude that the simple, rapid, less-time consuming, cost effective and precise method was developed and validated by UV-spectroscopy with the simultaneous estimation of simvastatin and ezetimibe.

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