

Development and Validation of UV-Spectrophotometric Method for Estimation of Simvastatin in Bulk and Solid Dosage Form.

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ABSTRACT

Simple, versatile, accurate, precise and economic method for UV spectrophotometric estimation of Simvastatin in bulk and tablet dosage form have been developed using absorbance ratio method and validated as per ICH guidelines. In present study the absorbance values at 238 were used for the estimation of simvastatin. The results of analyses have been validated statistically for linearity, accuracy, precision, LOD and LOQ. The method was found to be linear in concentration range of 5-50 µg/ml with recovery of 99.84% for simvastatin. The results of validation parameters also indicated that proposed method was found to be accurate, precise, reproducible, sensitive and suitable for routine quality control analysis for estimation of Simvastatin in bulk and solid dosage form.

Key Words: Simvastatin, Absorbance ratio method.

1. INTRODUCTION^{1,3}

Simvastatin (SMV) is chemically is 2,2-Dimethyl butanoic acid (1S,3R,7S,8S,8aR)-1,2,3,7,8,8ahexahydro- 3,7-dimethyl-8-[2-[(2R,4R)- tetrahydro-4- hydroxy-6 oxo2H pyran-2yl]ethyl]1-naphthalenyl ester used as a HMG-CoA reductase inhibitors. Simvastatin belongs to a class of drugs called HMG-CoA reductase inhibitors commonly called statins that derived synthetically from fermentation products of *Aspergillus terreus*. All statins act by inhibiting 3-hydroxy-3-methylglutarylcoenzyme (HMG-CoA).A HMG-CoA reductase, the rate limiting enzyme of the HMG-CoA reductase pathway, the metabolic pathway responsible for the endogenous production of cholesterol mainly used for the treatment of dyslipidemia and the prevention of cardiovascular diseases. Simvastatin is prodrug which is converted into its β- hydroxy which inhibits HMG CoA reductase (3-hydroxy-3-methyl glutarylCoenzyme A) enzyme, responsible for catalysing the conversion of HMG CoA to mevalonate a rate limiting step in the synthesis of cholesterol in liver.

There are various method available for the estimation of simvastatin such as HPLC, UPLC etc. The attempt of present study is was develop Simple, versatile, accurate, precise and economic method for UV spectrophotometric estimation of Simvastatin in bulk and tablet dosage form.

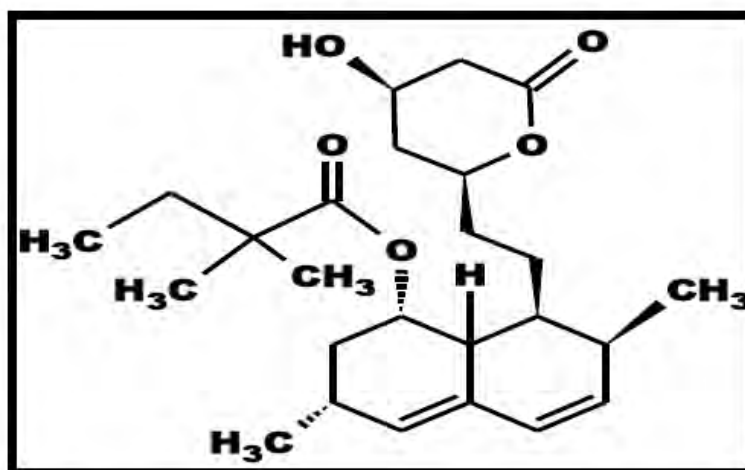


Fig.1.Chemical structure of Simvastatin

2. MATERIALS AND METHODS

2.1 Chemicals and reagents

The Simvastatin was obtained as free gift sample from FDC limited, Mumbai. All other chemical were analytical grade.

2.2 Instrumentation

UV-VIS Spectrophotometer, LABINDIA Model: UV-VIS Spectrophotometer, Number: 19-1885-01-0097, Spectral Bandwidth: 2.00 nm.

2.3 Preparation of standard stock solution

Standard stock solution of Simvastatin (100 μ g/mL) was prepared by weighing 10mg of Simvastatin and transferred to a 100 ml volumetric flask and volume was made up to 100 ml with ethanol & Water in the ratio of 40: 60(Ethanol: Water) to get a concentration of 100 μ g/ml, The prepared solution is sonicated for 15 minutes and filtered through the whatman filter paper. Appropriate volumes of this solution were further diluted to obtained final concentrations in range of 2 to 50 μ g/ml (table 1). The spectrum of this solution was recorded using LABINDIA Model: UV-VIS Spectrophotometer, Number: 19-1885-01-0097, Spectral Bandwidth: 2.00 nm in the range of 200-400 nm.

- **Preparation of solutions**

Table 1: Dilution table for calibration curve.

| Sr. No | Concentration in (ppm) | Stock solution in (ml) | Make up volume with ethanol (ml) |
|--------|------------------------|------------------------|----------------------------------|
| 1 | 2 | 0.2 | 9.8 |
| 2 | 4 | 0.4 | 9.6 |
| 3 | 6 | 0.6 | 9.4 |
| 4 | 8 | 0.8 | 9.2 |
| 5 | 10 | 1.0 | 9.0 |
| 6 | 15 | 1.5 | 8.5 |
| 7 | 20 | 2.0 | 8.0 |
| 8 | 25 | 2.5 | 7.5 |
| 9 | 30 | 3.0 | 7.0 |
| 10 | 35 | 3.5 | 6.5 |
| 11 | 40 | 4.0 | 6.0 |
| 12 | 45 | 4.5 | 5.5 |
| 13 | 50 | 5.0 | 5.0 |

2.4 Determination of Absorption maxima^{2, 3, 4, 6, 10}

For the selection of analytical wavelength, 25 μ g/ml solution of Simvastatin was scanned in the spectrum mode from 200 nm to 400 nm separately. From the spectra of drug, λ_{max} , 238nm was selected for the analysis (Fig. 2).

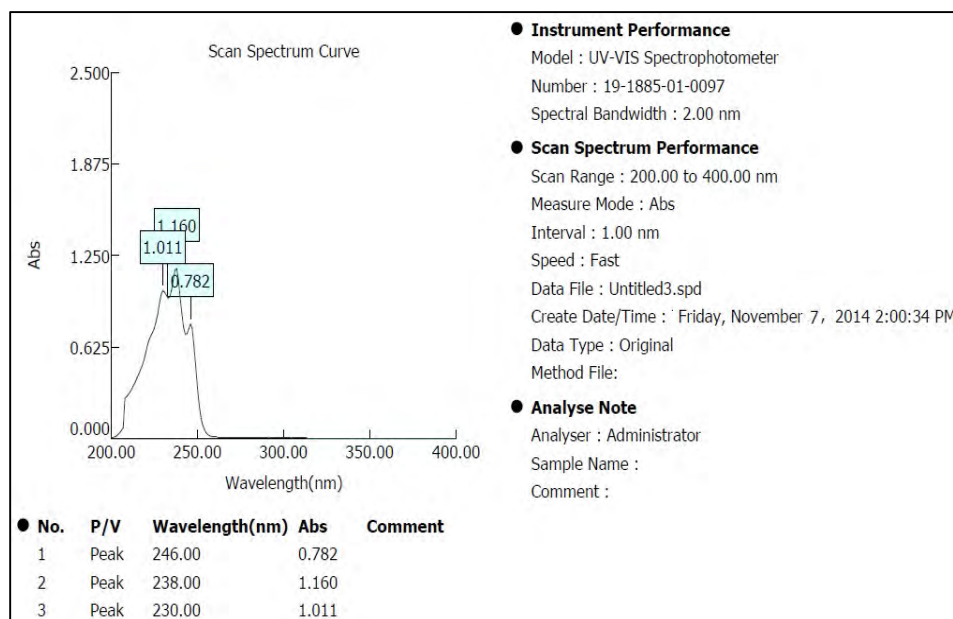


Fig.2. Standard calibration curve of simvastatin.

2.5 Validation^{4,5,8,9}

2.5.1 Linearity

The linearity of the analytical method is determined by taking concentration in between 2 to 50 µg/ml. From std. stock solution of Simvastatin (100 µg/ml), pipette out aliquots of 0.2 to 5.0 ml of solution transferred to series of 10 ml volumetric flasks and final volume made up to mark with Water as diluent to form solutions of 2 to 50 µg/ml of Simvastatin. These solutions were then scanned in the range of 200-400 nm against diluent as blank and then calibration curve was plotted as absorbance vs. concentration to check the linear relationship between absorbance and concentration of Simvastatin.

- **Acceptance Criteria:**

Correlation Coefficient should not be less than 0.999.

2.5.2 Precision

The precision of the system is determined by assay of six determinations at 100% test concentration (15ppm) and relative standard deviation (%RSD) is calculated. The results of precision study were reported in terms of % relative standard deviation.

- **Acceptance Criteria:**

The Relative Standard Deviation should not be more than 2%.

2.5.3 Accuracy

The accuracy of an analytical method is determined by applying the method to analyzed samples, to which known amounts of analyte have been added. The accuracy is calculated from the test results as the percentage of analyte recovered by the assay. The accuracy of developed method was carried out by calculating the % recovery of Simvastatin by standard addition method at three different levels i.e. 80 %, 100 % and 120 %.

- **Procedure for preparation of sample Solution:**

It was carried out at three levels i.e.80%, 100% and 120% of the nominal concentration.

- ❖ **80% Accuracy solution (8 ppm):**

It was prepared by diluting 0.8 ml of the stock solution up to 10ml with ethanol.

- ❖ **100% Accuracy solution (10 ppm):**

It was prepared by diluting 1.0 ml of the stock solution up to 10ml with ethanol.

- ❖ **120% Accuracy solution (12 ppm):**

It was prepared by diluting 1.2 ml of the stock solution up to 10ml with ethanol.

The sample was prepared in triplicate and analyzed by using U.V. spectrophotometer at wavelength 238 nm.

- **Acceptance Criteria:**

Mean recovery should be in the range of 98-102%.

2.5.4 Limit of detection

It is the lowest amount 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 guidelines.

$$\text{LOD} = 3.3 \times \sigma / S$$

Where,

σ = Standard deviation of the response and

S = Slope of the corresponding calibration curve.

2.5.5 Limit of quantification

It is the lowest concentration of analyte in a sample that can be determined with the acceptable precision and accuracy under stated experimental conditions. Limit of quantification can be calculated using following equation as per ICH guidelines.

$$\text{LOQ} = 10 \times \sigma / S$$

Where,

σ = Standard deviation of the response and

S = Slope of the corresponding calibration curve.

3. RESULTS AND DISCUSSION

3.1 Method development and optimization

The present paper describes the application of absorption ratio method to estimation of simvastatin in bulk and solid dosage form. The method were validated for the linearity, accuracy, precision, LOD and LOQ.

3.2 Validation

3.2.1 Linearity

The calibration curve was taken in the range of 2-50 $\mu\text{g/ml}$ for Simvastatin at λ_{max} 238nm. The Simvastatin was found to be linear within concentration range of 2-50 $\mu\text{g/ml}$ with regression coefficient of 0.9992. by absorbance ratio method. There was an excellent correlation between absorbance and concentration.

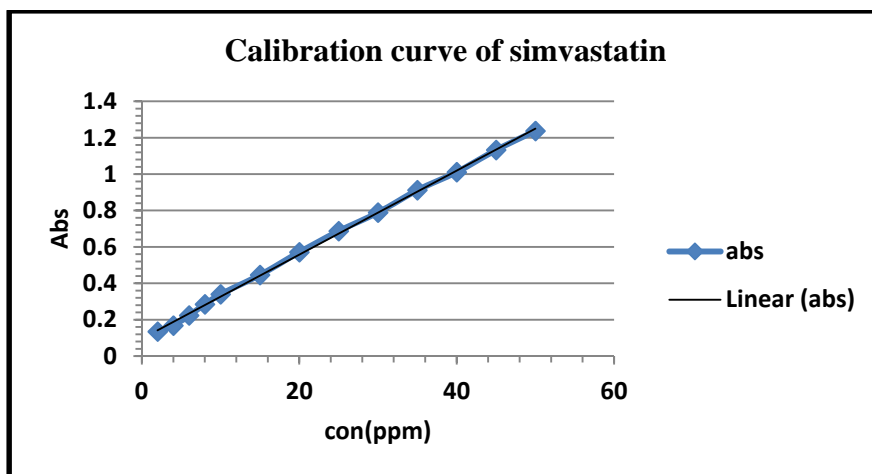


Fig. 3: Calibration curve of Simvastatin in ethanol

3.2.2 Precision

The precision of an analytical method is determined by assaying six determinations at test concentration (15ppm). % Relative Standard Deviation (%RSD) calculate statistically. It was found to be less than 2%. (i.e.0.24%)

Table 2: Calculation for precision

| Sr. No. | Solution (ppm) | Abs. (nm) | Avg. | SD | %RSD (%) |
|---------|----------------|-----------|-------|--------|----------|
| 1 | 15 | 0.571 | 0.570 | 0.0013 | 0.24 |
| 2 | 15 | 0.573 | | | |
| 3 | 15 | 0.570 | | | |
| 4 | 15 | 0.569 | | | |
| 5 | 15 | 0.572 | | | |
| 6 | 15 | 0.570 | | | |

3.2.3 Accuracy

The accuracy was assessed by the standard addition method of three replicate determinations of three different solutions containing 8, 10 and 12 µg/ml (i.e.80%, 100% and 120% of the nominal concentration) of simvastatin. The average % recoveries for three different concentrations was found to be 99.34%.

Table 3: Calculation for accuracy

| Sr. No. | Sample Identity | Conc. (PPM) | Abs. | Mean Abs. | Amount Recovered in ppm | % recovered | Average % recovered |
|---------|---------------------|-------------|-------|-----------|-------------------------|-------------|---------------------|
| 1 | Accuracy 80% set-1 | 8 ppm | 0.221 | 0.222 | 7.82 | 97.75 | 99.84 |
| | | 8 ppm | 0.224 | | | | |
| | | 8 ppm | 0.223 | | | | |
| 2 | Accuracy 100% set-1 | 10 ppm | 0.285 | 0.285 | 9.91 | 99.1 | |
| | | 10 ppm | 0.284 | | | | |
| | | 10 ppm | 0.286 | | | | |
| 3 | Accuracy 120% set-1 | 12 ppm | 0.312 | 0.313 | 12.32 | 102.66 | |
| | | 12 ppm | 0.315 | | | | |
| | | 12 ppm | 0.313 | | | | |

LOD and LOQ

The overlain spectra of both the drugs showed that the peaks are well resolved, thus satisfying the criteria for obtaining maximum precision, based on absorbance ratio. The limit of detection was found to be 0.15µg/ml and the limit of quantification was found to be 2.37µg/ml. The criteria being the concentration should lie outside the range 0.1 – 2.0 for precise determination of simvastatin.

4. CONCLUSION

Simple, economic, fast and non-toxic UV spectrophotometric methods have been developed for the estimation of Simvastatin. The methods also validated as per ICH guidelines for linearity, precision, accuracy, LOD and LOQ. The results of these all parameters were shows that the present UV spectrophotometric methods found to be linear, precise and accurate and can be used for routine quality control analysis of Simvastatin in bulk and tablet dosage form.

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