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# DEVELOPMENT AND VALIDATION OF SPECTROSCOPY METHOD FOR SIMVASTATIN IN DIFFERENT DISSOLUTION MEDIA

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## Abstract

The aim of present work is to develop and validate simple, sensitive and specific spectrophotometric method for the determination of Simvastatin, a hypolipidemic drug in pure form and in pharmaceutical formulations. UV spectrophotometric method, which is based on measurement of absorption at maximum wavelength in solvent system employed for the determination of simvastatin was methanol with 0.05% acetic acid, water with 0.03% w/v of SLS, 1.2 pH with 0.03% w/v of SLS, phosphate buffer pH 6.8 and 7.4 pH with 0.03% w/v of SLS, was found to be at 238 nm. The developed method was validated with respect to linearity, accuracy (recovery), precision and specificity. The optimum conditions for analysis of the drug were established. The drug obeyed the Beer's law and showed good correlation. Beer's law was obeyed in concentration range 0-16 µg/ml. The results of analysis were validated by recovery studies. The method was found to be simple, accurate, precise, economical and robust. This method was extended to formulation and there was no interference from excipients and diluents. This method has been statistically validated and is found to be precise and accurate.

Results obtained that are further used for the formulation development.

## 1. 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 b-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 tomevalonate, which is an early and rate limiting step in cholesterol biosynthesis[2] .Ezetimibe (EZ), 1-(4-Fluorophenly) – 3 (R) - [3-(4-fluorophenyl) - 3 (S) hydroxyl 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]. This distinct mechanism of action results in a synergistic cholesterol lowering effect when used together with statins that inhibits cholesterol synthesis by liver[4].

Simvastatin is rapidly absorbed from the gastrointestinal tract after oral administration but undergoes extensive first-pass metabolism in the liver. It is inactive lactone prodrug and hydrolyzed in the gastrointestinal tract to the active ß hydroxy derivative (Figure 2).

SIM may be determined by several methods including gas chromatography–mass spectrometry (GC–MS)[6], liquid chromatography with UV detection (LC–UV)[7-9]. Literature survey revealed that there is few UV-visible methods have been reported.

## 2. EXPERIMENTAL

### Instrumentation

The present work was carried out on UV-1700, Shimadzu Corporation, Japan 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 200-400 nm.

### Chemicals:

All the solvents and reagents used were of analytical grade

**3. METHOD**

All chemicals of analytical grade used as it is. Preparation of standard solution A stock solution of 1 mg/ml was prepared in methanol with 0.05% acetic acid. Interpenetrating polymeric network (IPN) hydrogel beads prepared by precipitation method was assay for amount of drug present by dissolving a 10 mg equivalent amount of drug was determined by, suitably diluted with methanol and UV absorbance was measured at 238 nm. Drug concentration was determined from standard graph of drug.

**Analytical Method Development:** [10,11]

A UV spectrophotometry method was developed and calibration curve constructed in the different modified solvent systems. In Methanol with 0.05%w/v acetic acid and in water with 0.03% w/v Sodium lauryl sulphate (SLS) were used for measurement of drug content of formulation and phase solubility of drug in β- cyclodextrin respectively. While 1.2 pH, 6.8pH, and 7.4 pH with 0.03% w/v SLS buffer were used for dissolution purpose.

**Preparation of various buffer solutions as per IP'96:**

- ***Methanol with 0.05%w/v acetic acid*:** Add 0.5ml of glacial acetic acid in 1000ml of methanol
- ***Hydrochloric acid solution, 0.1 N (pH 1.2)*:** Concentrated hydrochloric (8.5 ml) acid was diluted with distilled water and volume was made up to 1000 ml with distilled water. pH (1.2) was adjusted with dilute hydrochloride.
- ***0.03% SLS solution in 0.1N HCl (pH 1.2):*** Dissolve 0.3gm of Sodium lauryl Sulphate in 1000ml 0.1N HCl (pH 1.2) and adjust the pH with 0.1N HCl
- ***Phosphate Buffer pH 6.8*:** Dissolve 28.80 g of disodium hydrogen phosphate and 11.45 g of potassium dihydrogen phosphate in sufficient water to produce 1000 ml.
- ***0.03% SLS solution in pH 6.8***: Dissolve 0.3gm of Sodium lauryl Sulphate in 1000ml pH 6.8
- ***Phosphate Buffer pH 7.4*:** Dissolve 2.38 g of disodium hydrogen

phosphate, 0.19 g of potassium dihydrogen phosphate and 8.0 g sodium chloride in sufficient water to produce 1000 ml.

- ***0.03% SLS solution in pH 7.4***: Dissolve 0.3gm of Sodium lauryl Sulphate in 1000ml pH 7.4

**UV spectrophotometric method of Simvastatin**

Here different buffer was selected based on different dissolution media used for the IPN hydrogel bead formulation development.

***Scanning of Simvastatin in different buffer***

50 mg of Simvastatin was accurately weighed and dissolved in 50 ml of methanol with 0.05%w/v acetic acid,1.2 pH with 0.03%w/v SLS, 6.8pH with 0.03%w/v SLS , and 7.4 pH with 0.03%w/v SLS to get stock solution I, II, III and IV respectively of 1000 µg/ml. Further primary standard solution V, VI, VII, and VIII were prepared from the stock to get a concentration of 50 µg/ml. The aliquot was scanned in the wavelength range of 200 to 400 nm on Shimadzu UV spectrophotometer to determine the wavelength of maximum absorbance. The solution was found to exhibit one absorption maxima at 238 nm.

***Calibration curve for Simvastatin in different buffer***

- **Preparation of standard solution:**

An aliquot of 5 ml of the stock solution (I, II, III and IV) was diluted to 50 ml to get a standard solution-V, VI, VII, and VIII having a concentration of 100 µg/ml using methanol with 0.05%w/v acetic acid, 1.2 pH with 0.03%w/v SLS, 6.8pH with 0.03%w/v SLS, and 7.4 pH with 0.03%w/v SLS respectively.

- **Preparation of the working standard solution:**

Working standard solutions having concentration of 2 to 16µg/ml were prepared by appropriately diluting the standard solution V, VI, VII, and VIII with respective buffer. The absorbance of each working standard solution was measured at 238nm in Shimadzu UV spectrophotometer using respective buffer as a blank. Average of triplicate readings was taken and tabulated. Beer's law was obeyed in the range of 2-16 µg/ml as a linear plot was obtained when absorbance were plotted against

concentration using Microsoft Excel®. Regression equation was derived from the slope of the curve.

**4. RESULTS AND DISCUSSION:**

**A. Analytical Method Development**

In order to ascertain the wavelength of maximum absorption (λ max) of the drug, working standard solution of the drug (10µg/ml) in methanol with 0.05%w/v acetic acid, 1.2 pH with 0.03%w/v SLS, 6.8pH with 0.03%w/v SLS, and 7.4 pH with 0.03%w/v SLS was scanned using UV-VIS spectrophotometer within the wavelength region of 200 – 400 nm against solvent blank. The resulting spectra are shown in Figure below and the absorption curve for all solvent shows characteristic absorption maxima at 238nm for Simvastatin.

***1. Scanning of Simvastatin in Methanol with 0.05%w/v acetic acid:***

***1.1 Calibration curve of Simvastatin in Methanol with 0.05%w/v acetic acid at $\lambda_{max}$ 238nm.***

The solutions of SIM in Methanol with 0.05%w/v acetic acid were found to exhibit absorption maxima at 238nm(Figure3). Beer's law was found to be obeyed in the concentration range of 0-16 µg/ml (Table1) as a linear plot ($R^2$ = 0.998) was obtained when absorbance values of the working standard solutions was plotted against concentration as shown in Figure 4.

***2. Scanning of Simvastatin in water with 0.03% SLS:***

***2.1 Calibration curve of Simvastatin in water with 0.03%w/v SLS at $\lambda_{max}$***

The solutions of SIM in water with 0.03%w/v SLS were found to exhibit absorption maxima at 238nm (Figure 5). Beer's law was found to be obeyed in the concentration range of 0-16 µg/ml (Table2**)** as a linear plot ($R^2$ = 0.997) was obtained when absorbance values of the working standard solutions was plotted against concentration as shown in Figure 6.

***3. Scanning of Simvastatin in 1.2 pH with 0.03% SLS:***

***3.1 Calibration curve of Simvastatin in 1.2 pH with 0.03%w/v SLS at $\lambda_{max}$***

The solutions of SIM in 1.2 pH with 0.03%w/v SLS were found to exhibit absorption maxima at 238nm (Figure7).

Beer's law was found to be obeyed in the concentration range of 0-16 µg/ml (Table3) as a linear plot ($R^2$ = 0.998) was obtained when absorbance values of the working standard solutions was plotted against concentration as shown in Figure 8.

***4. Scanning of Simvastatin in 6.8 pH with 0.03% SLS:***

***4.1 Calibration curve of Simvastatin in 6.8pH phosphate buffer with 0.03%w/v SLS at $\lambda_{max}$***

The solutions of SIM in 6.8 pH with 0.03%w/v SLS were found to exhibit absorption maxima at 238nm (Figure 9). Beer's law was found to be obeyed in the concentration range of 0-16 µg/ml (Table4) as a linear plot ($R^2$ = 0.998) was obtained when absorbance values of the working standard solutions was plotted against concentration as shown in Figure 10.

**Table 4: Absorbance values of working Standard solution Simvastatin in 6.8 pH with 0.03%w/v SLS**

***5. Scanning of Simvastatin in phosphate buffer pH 7.4 with 0.03% SLS:***

***5.1 Calibration curve of Simvastatin in 7.4pH phosphate buffer with 0.03%w/v SLS at $\lambda_{max}$***

The solutions of SIM in 7.4 pH with 0.03%w/v SLS were found to exhibit absorption maxima at 238nm (Figure11). Beer's law was found to be obeyed in the concentration range of 0-16 µg/ml(Table5) as a linear plot ($R^2$ = 0.998) was obtained when absorbance values of the working standard solutions was plotted against concentration as shown in Figure 12.

***6. Scanning of Simvastatin in methanol with 0.05% acetic acid for IPN hydrogel bead.***

**B. Determination of Absorptivity Value, A (1%, 1cm) of Simvastatin at selected Wavelength:**

Accurately measured 1 ml of stock solution was added to 10 ml volumetric flask and volume was made up to the mark with methanol to get final concentration of 10µg/ml of Simvastatin five dilutions. Absorbance for each dilution was measured at 238nm against solvents blank and absorptivity values were calculated.

**C. Estimation of Simvastatin in Interpenetrating Polymeric Network (IPN) Hydrogel Bead Formulation:**

The proposed method was applied to analyse on Interpenetrating polymeric network (IPN) hydrogel bead. The amount of powder equivalent to 5 mg of Simvastatin was weighed accurately and transfer to 50ml volumetric flask containing 5ml 0.05% acetic acid and shake until beads were dissolve, then finally adjust volume up to 50ml with methanol and kept for 15 min for ultrasonication, and the volume was made up to the mark with methanol. The solution was then filtered through Whattman filter paper, 1ml of the filtrate was diluted suitably with solvent up to 10 ml to get the solution of 10µg/ml concentration .The absorbance was measured against solution blank. The % Drug content % Drug entrapment of Simvastatin was calculated by using following equation:

$$\text{Drug content (\%)} = \frac{\text{Amount of drug in IPN hydrogel beads}}{\text{Amount of drug taken}} \times 100 \quad \ldots\ldots (1)$$

The amount of drug entrapped was calculated by using following equation: **(**Das MK., 2007)

$$\text{\% Drug entrapment} = \frac{\text{Experimental drug content}}{\text{Theoretical drug content}} \times 100 \quad \ldots\ldots (2)$$

**D. Method Validation (as per ICH guidelines) [12]:**

***1. Accuracy:***

Sample Solutions were prepared at by adding 80%, 100% and 120% pure drug as per the test method dilution was made as according to and absorbance of each solutions were taken .The recovery result showed that the proposed method has an acceptable level of accuracy for Simvastatin.

***2.Precision***

The IPN hydrogel beads of samples of Simvastatin and working standard of concentration 10µg/ml was prepared and precision studies analysed by using different analysts , different days.

**2.1 Different analyst**

**2.2 Different days**

***3. Linearity Range:***

The linearity of the response of the drug was verified at 0 to 16 µg/ml concentrations. The calibration curve was obtained by plotting the absorbance versus the concentration data and was treated by linear regression analysis .The calibration curve was found to be linear in y = 0.0565x - 0.0032 ($R^2$ = 0.9985) in Methanol with 0.05%w/v acetic acid , y = 0.0567x + 0.0032 ($R^2$ = 0.9972) in water with 0.03%w/v SLS, y = 0.0434x + 0.0038 ($R^2$ = 0.9983) in 1.2 pH with 0.03%w/v SLS, y = 0.046x - 0.0017 ($R^2$ = 0.9989) in 6.8 pH with 0.03%w/v SLS, y = 0.0442x + 0.0044 ($R^2$ = 0.9981) in 7.4 pH with 0.03%w/v SLS at $\lambda_{max}$ 238nm for aforementioned concentrations.

***4. Robustness:***

Robustness study was carrying out by change in wavelength (± 2 nm):

**5.CONCLUSION:**

The developed method was found to be simple, sensitive, accurate, precise, reproducible, and can be used for routine quality control analysis of Simvastatin in bulk and pharmaceutical formulation.

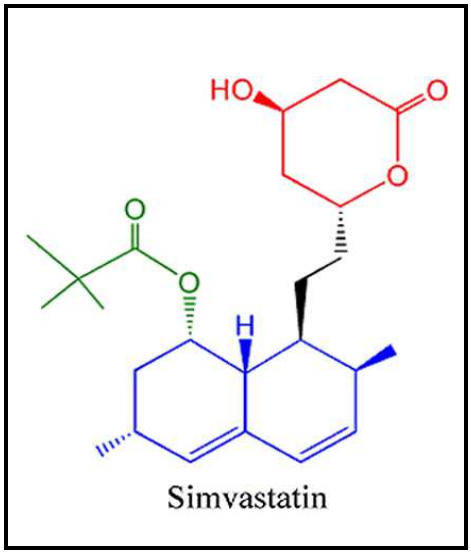


**Figure 1: Chemical structure of simvastatin**

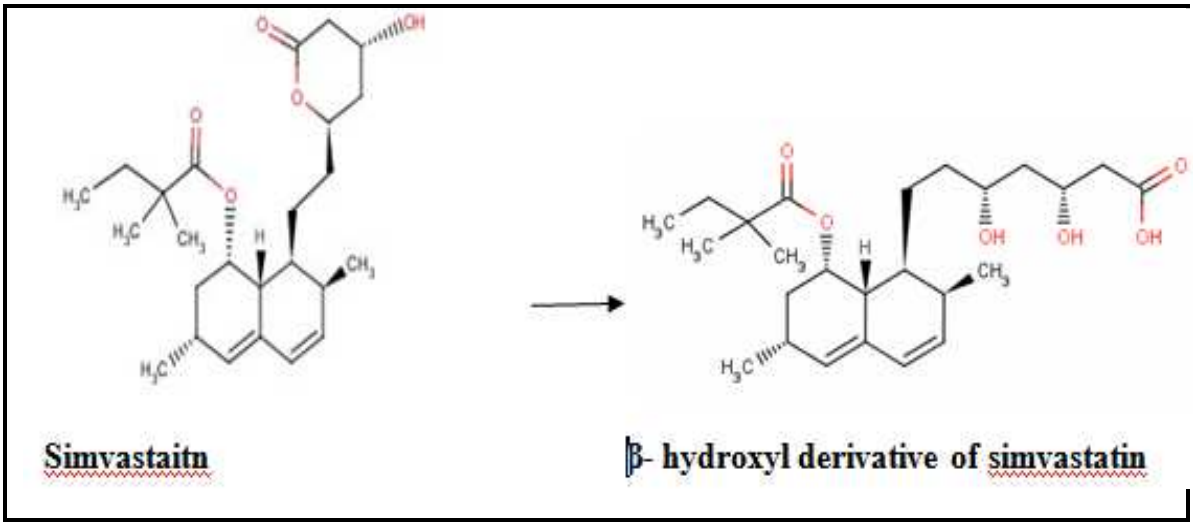


**Figure 2: Conversion of Simvastatin to its active metabolites[2]**

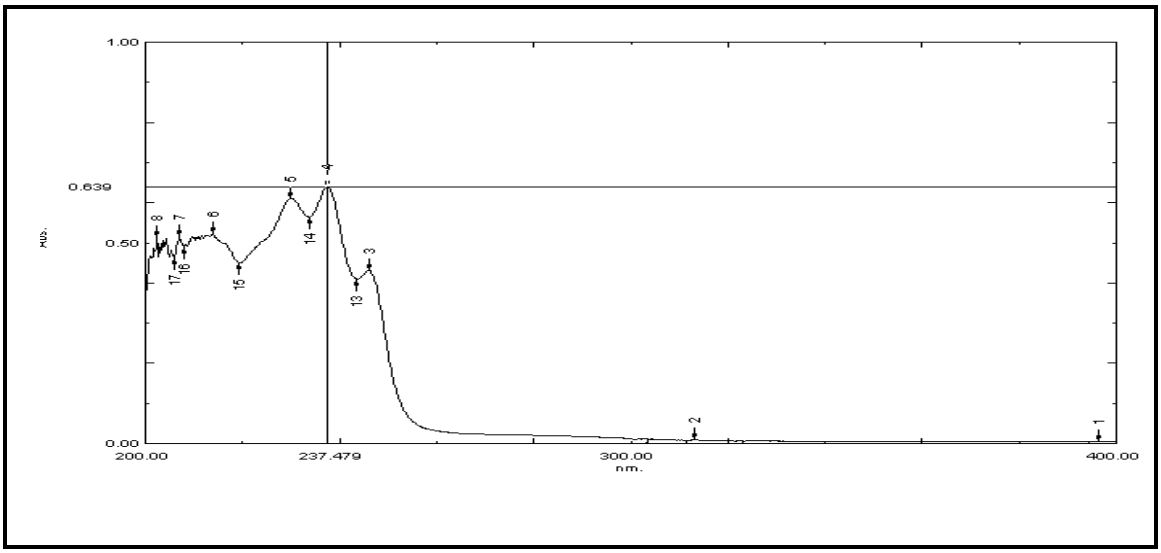


**Figure 3: UV spectrum of Simvastatin in Methanol with 0.05%w/v acetic acid**

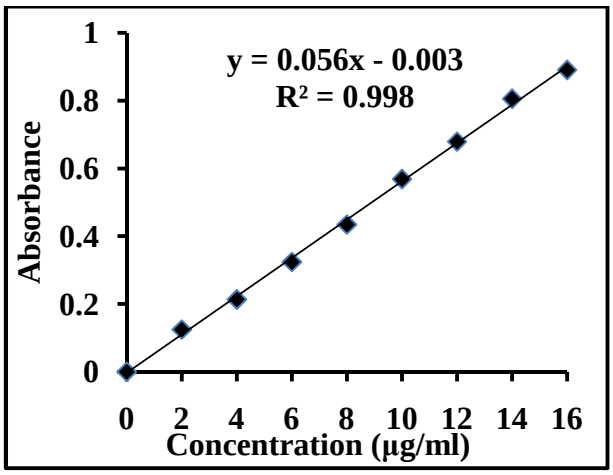


**Figure 4: Calibration curve of Simvastatin in Methanol with 0.05%w/v acetic acid at $\lambda_{max}$ 238nm**

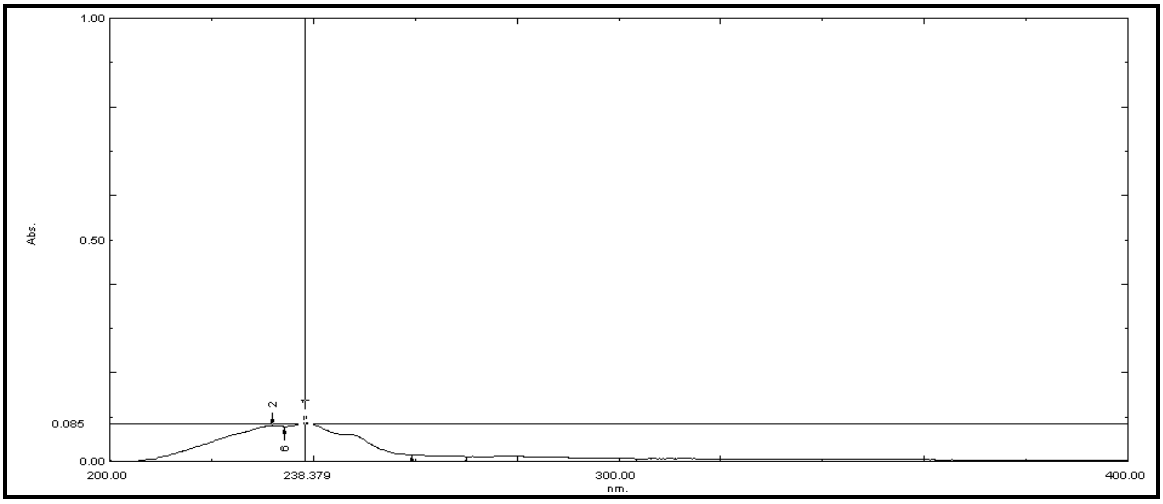


**Figure 5: UV spectrum of Simvastatin in water with 0.03% SLS**

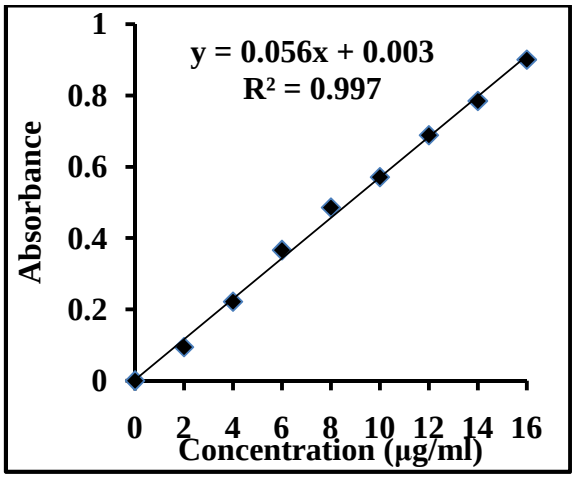


**Figure 6: Calibration curve of Simvastatin in water with 0.03%w/v SLS at $\lambda_{max}$ 238nm**

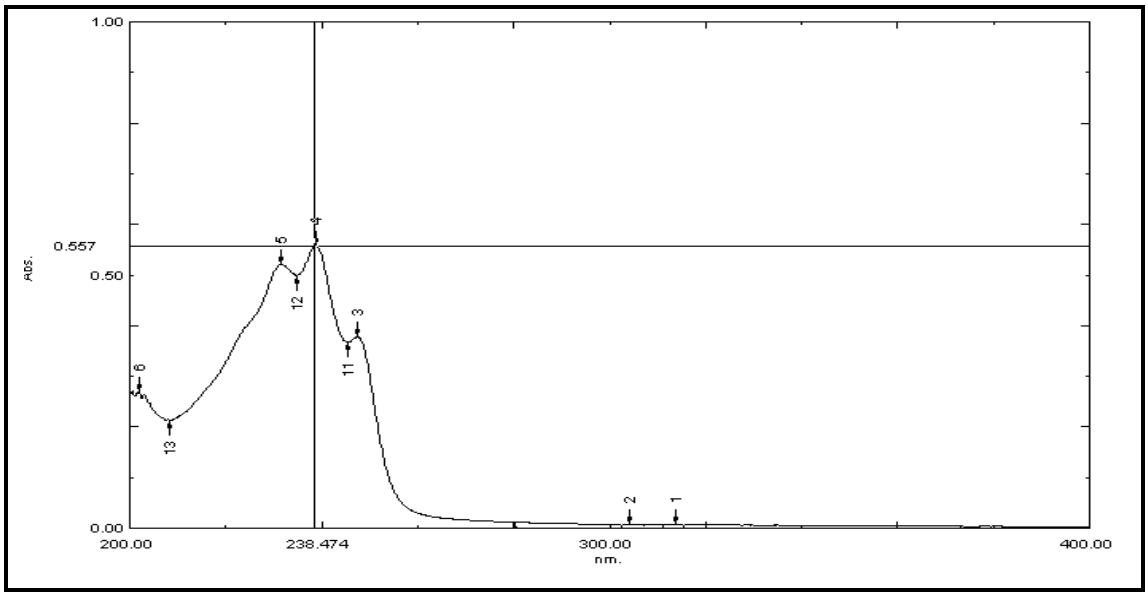


**Figure 7: UV spectrum of Simvastatin in 1.2 pH with 0.03% SLS**

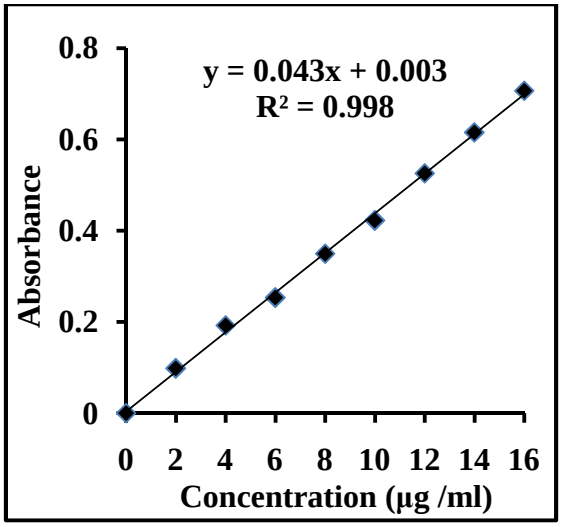


**Figure 8: Calibration curve of Simvastatin in 1.2 pH with 0.03%w/v SLS at $\lambda_{max}$ 238nm**

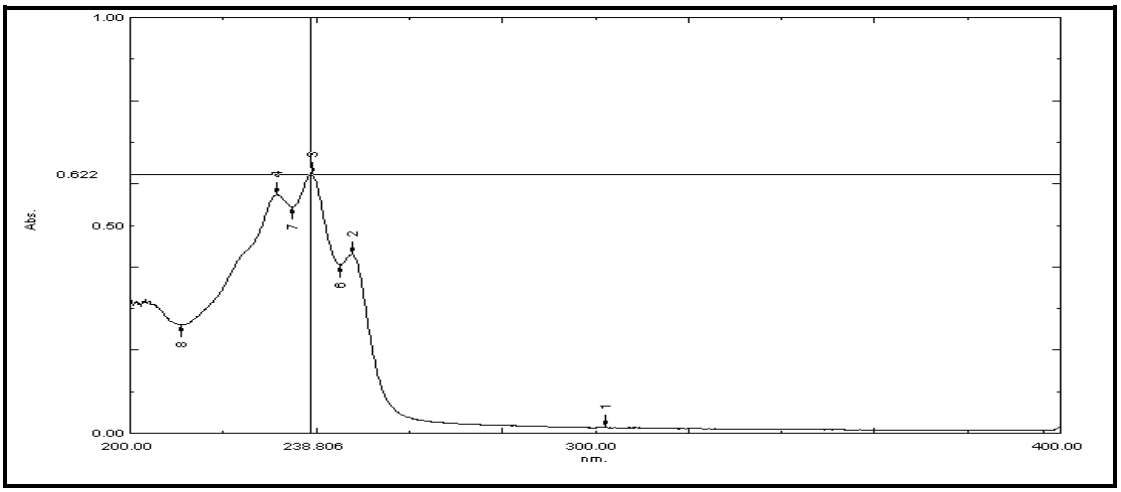


**Figure 9: UV spectrum of Simvastatin in phosphate buffer 6.8 pH with 0.03% SLS**

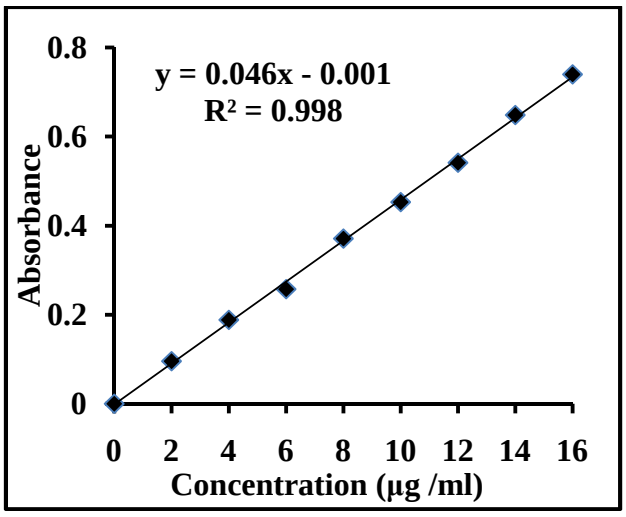


**Figure 10: Calibration curve of Simvastatin in 6.8 pH with 0.03%w/v SLS at $\lambda_{max}$ 238nm**

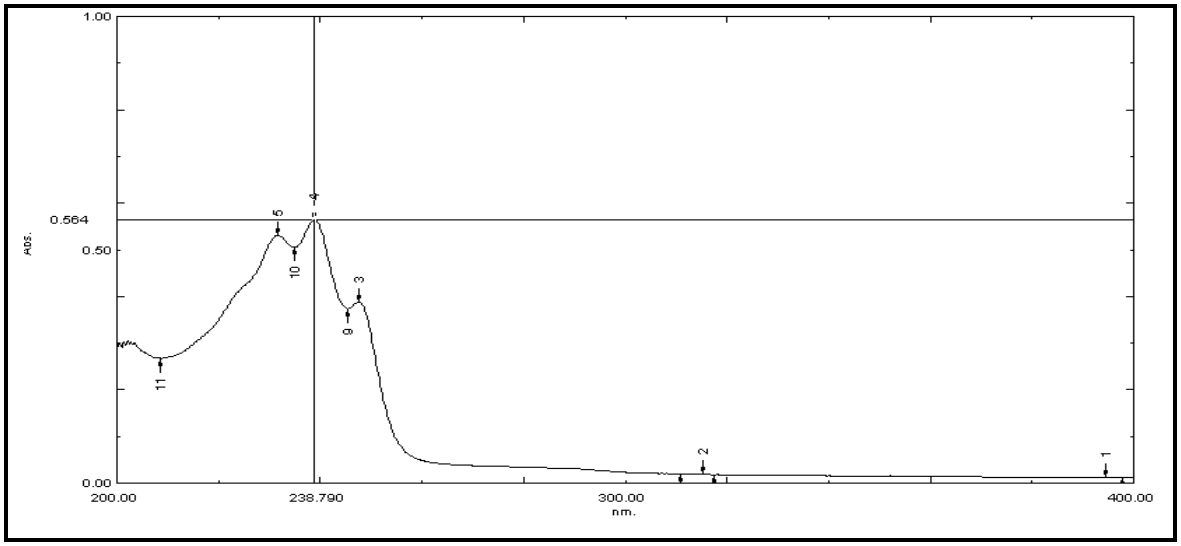


**Figure 11: UV spectrum of Simvastatin in pH 7.4 with 0.03% SLS**

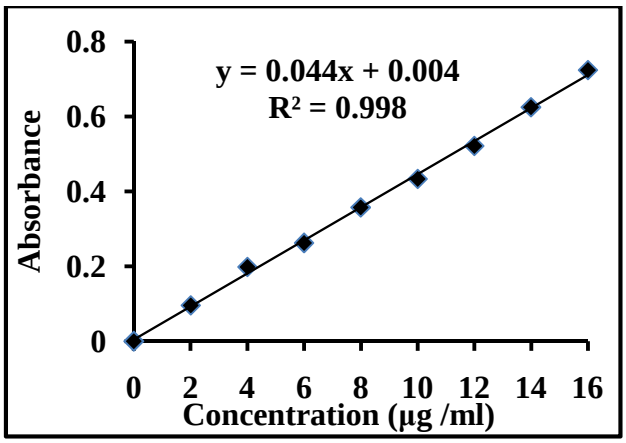


**Figure 12: Calibration curve of Simvastatin in 7.4 pH with 0.03%w/v SLS at $\lambda_{max}$ 238nm**

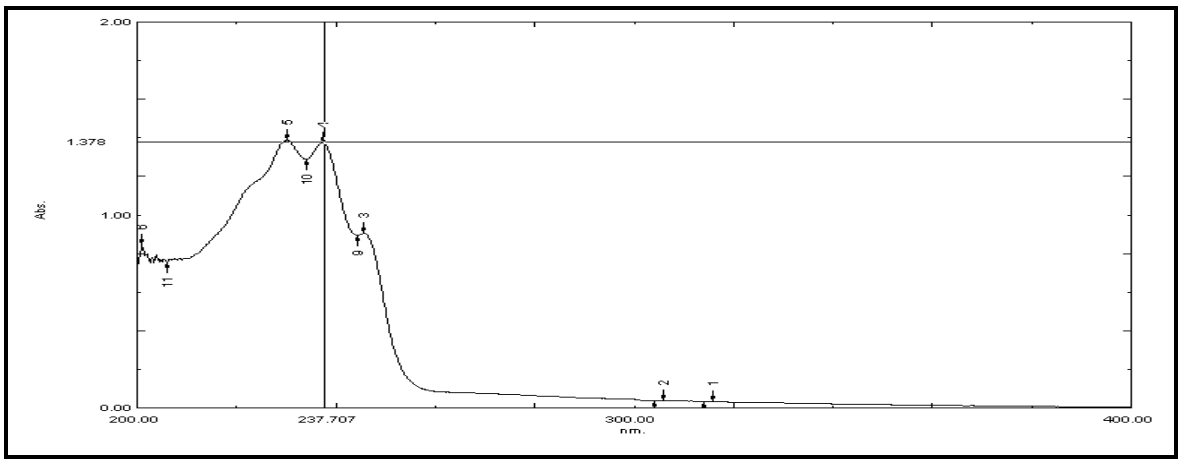


**Figure 13: UV spectrum of Simvastatin in methanol with 0.05% acetic acid for IPN hydrogel bead**

**Table 1: Absorbance values of Working Standard solution Simvastatin in Methanol with 0.05%w/v acetic acid**

| Concentration in µg /ml | Absorbance at 238 nm, n=3 |
|---|---|
| 0 | 0 |
| 2 | 0.125±0.004 |
| 4 | 0.213±0.001 |
| 6 | 0.324±0.012 |
| 8 | 0.435±0.012 |
| 10 | 0.568±0.008 |
| 12 | 0.679±0.009 |
| 14 | 0.805±0.051 |
| 16 | 0.890±0.004 |

**Table 2: Absorbance values of Working Standard solution of Simvastatin in water with 0.03% w/v SLS**

| Concentration in µg /ml | Absorbance at 238 nm, n=3 |
|---|---|
| 0 | 0 |
| 2 | 0.095±0.002 |
| 4 | 0.222±0.006 |
| 6 | 0.366±0.009 |
| 8 | 0.486±0.006 |
| 10 | 0.571±0.009 |
| 12 | 0.688±0.003 |
| 14 | 0.785±0.007 |
| 16 | 0.900±0.005 |

**Table 3. Absorbance values of working Standard solution Simvastatin in 1.2 pH with 0.03%w/v SLS**

| Concentration in µg /ml | Absorbance at 238 nm, n=3 |
|---|---|
| 0 | 0 |
| 2 | 0.098±0.001 |
| 4 | 0.192±0.003 |
| 6 | 0.253±0.003 |
| 8 | 0.349±0.003 |
| 10 | 0.422±0.004 |
| 12 | 0.525±0.004 |
| 14 | 0.615±0.004 |
| 16 | 0.707±0.005 |

**Table 4: Absorbance values of working Standard solution Simvastatin in 6.8 pH with 0.03%w/v SLS**

| Concentration in µg /ml | Absorbance at 238 nm, n=3 |
|---|---|
| 0 | 0 |
| 2 | 0.096±0.002 |
| 4 | 0.189±0.003 |
| 6 | 0.258±0.004 |
| 8 | 0.371±0.004 |
| 10 | 0.453±0.006 |
| 12 | 0.541±0.005 |
| 14 | 0.649±0.003 |
| 16 | 0.740±0.002 |

**Table 5: Absorbance values of working Standard solution of Simvastatin in 7.4 pH with 0.03%w/v SLS**

| Concentration in µg /ml | Absorbance at 238 nm, n=3 |
|---|---|
| 0 | 0 |
| 2 | 0.096±0.002 |
| 4 | 0.199±0.004 |
| 6 | 0.262±0.005 |
| 8 | 0.358±0.002 |
| 10 | 0.434±0.005 |
| 12 | 0.521±0.009 |
| 14 | 0.625±0.005 |
| 16 | 0.724±0.005 |

**Table 6: A (1%, 1cm) of Simvastatin at 238 nm**

| Sr.No | A(1%, 1 cm)* |
|---|---|
| **1** | 565.3 |
| **2** | 563.4 |
| **3** | 564.2 |
| **4** | 563.6 |
| **5** | 563.2 |
| MEAN | 563.94 |
| S.D | 0.847 |

**Table 7: Results of estimation of % Drug entrapment of Simvastatin IPN hydrogel bead.**

| Sr. No. | Standard Concentration | Absorbance at 238nm | | % Drug entrapment |
|---|---|---|---|---|
| | | **Standard** | **Sample** | |
| **1** | 10 | 0.561 | 0.568 | 101.25 |
| **2** | 10 | 0.561 | 0.569 | 101.43 |
| **3** | 10 | 0.561 | 0.565 | 100.71 |
| **4** | 10 | 0.561 | 0.558 | 99.47 |
| **5** | 10 | 0.561 | 0.559 | 99.64 |
| | | | **Mean** | **100.50** |
| | | | **± S.D** | **0.90** |

* Each value is mean of five observations

**Table 8: Results of Recovery studies**

| Sr. No | Std. Conc. (µg/ml) | Absorbance at 238nm | | % Drug entrapment |
|---|---|---|---|---|
| | | **Standard** | **Sample** | |
| **1** | 10 | 0.561 | 0.547 | 97.50446 |
| **2** | 10 | 0.561 | 0.574 | 102.3173 |
| **3** | 10 | 0.561 | 0.583 | 103.9216 |
| | | | **Mean** | 101.2478 |
| | | | **± S.D** | 0.543 |

* Each value is mean of five observations

**Table 9: Results of precision studies using different analysts**

| Sr . No. | Different Analysts | % Drug entrapment |
|---|---|---|
| **1** | Analyst 1 | 99.12 |
| **2** | Analyst 2 | 99.55 |
| **3** | Analyst 3 | 100.56 |
| | **Mean** | 99.74333 |
| | **± S.D** | 0.739211 |

* Each value is mean of five observations

**Table 10: Results of precision studies in different days**

| Sr. No | Observations | % Drug Estimation | |
|---|---|---|---|
| | | **Interday** | **Intraday** |
| **1** | **I** | 100.10 | 99.85 |
| **2** | **II** | 100.68 | 99.93 |
| **3** | **III** | 99.34 | 99.17 |
| | **Mean** | 100.04 | 99.65 |
| | **± S.D** | 0.572 | 0.517 |

* Each value is mean of five observations

**Table 11 Results of Robustness study**

| Sr . No. | Wavelength (± 2 nm) | % Drug Estimation |
|---|---|---|
| **1** | 236 nm | 99.51 |
| | | 99.26 |
| **2** | 238 nm | 99.26 |
| | | 100.50 |
| **3** | 240 nm | 100.74 |
| | | 99.26 |
| | **Mean** | 99.79 |
| | **± S.D** | 0.6515 |

*Each value is mean of five observations

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