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Original Article

# METHOD VALIDATION OF SIMVASTATIN IN PCL-PEG-PCL TRIBLOCK COPOLYMER MICELLES USING UV-VIS SPECTROPHOTOMETRIC FOR SOLUBILITY ENHANCEMENT ASSAY

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**ABSTRACT**

**Objective:** This study aims to increase the solubility of simvastatin (SIM), a hydrophobic drug, by incorporating it into PCL-PEG-PCL triblock copolymer micelles and validating the assay method used, namely Uv-Vis spectrophotometric.

**Methods:** The shake flask method was used to determine the increase in solubility experienced by SIM after being incorporated into the micellar system. The values of maximum wavelength ($\lambda_{max}$), linearity, LOD, LOQ, accuracy, and precision were used as parameters measured to assess the validity of the assay method used.

**Results:** The results showed that PCL-PEG-PCL triblock copolymer micelles could increase SIM solubility by 9.7 times (89.49±5.75 µg/ml) compared to SIM without modification (9.19±0.24 µg/ml). The validation results show the $\lambda_{max}$ value of 239 nm, a linear calibration curve with an R-value of 0.9994, LOD and LOQ of 0.33 µg/ml and 1.00 µg/ml, accurate measurement with recovery at concentrations of 80%, 100%, and 120% were 102.93±1.32%, 100.78±0.40%, and 104.58±0.79% and also had good precision with RSD<2%.

**Conclusion:** The PCL-PEG-PCL triblock copolymer micelles can increase SIM solubility and the Uv-Vis spectrophotometric method has been validated successfully for the quantitative analysis of SIM in PCL-PEG-PCL triblock copolymer micelles.

**Keywords**: Simvastatin, Triblock copolymer, PCL, PEG, Validation, Uv-Vis spectrophotometric


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## INTRODUCTION

Solubility is one of the physicochemical properties of drugs that need to be considered because it can affect the formulation and effectiveness of therapy. Drugs with low solubility (hydrophobic drugs) will provide low bioavailability so that the desired therapeutic effect is not perfect [1, 2].

SIM ($C_{25}H_{38}O_5$) is an anticholesterol drug of the statin class with the mechanism of action of inhibiting the enzyme 3-hydroxy-3-methyl glutaryc-coenzyme A reductase (HMG-CoA reductase). SIM belongs to the class II biopharmaceutical classification system (BCS) with a solubility of 0.01 g/l (practically insoluble) and a bioavailability of<5% [3-6].

Many attempts have been made to increase the solubility of SIM, including hydrogels [7], complexes with arginine [8], solid dispersions [9, 10], micellar polymers with derivatives of tocopherol [11], spherical crystal [12], and co-crystal formation [13, 14]. In this study, the increase in the solubility of SIM was carried out by being incorporated into PCL-PEG-PCL triblock copolymer micelles which would then form a micellar polymer. PCL-PEG-PCL triblock copolymer micelles are an ideal drug carrier candidate for SIM with an entrapment efficiency of 87.74% [15].

To determine the increase in solubility experienced by SIM, it is necessary to determine the concentration of SIM in the PCL-PEG-PCL triblock copolymer micelle. According to the pharmacopeia, SIM levels were determined by the High-Performance Liquid Chromatography (HPLC) method. However, a simpler method, UV-Vis spectrophotometry, has been reported to be used for the assay of SIM in several pharmaceutical preparations showing results that meet the required acceptance criteria [16-19].

## MATERIALS AND METHODS

### Materials

SIM is provided free by Dexa Medica (Palembang-Indonesia). All other chemicals and reagents used in this study met the criteria for an analytical grade.

### Preparation of PCL-PEG-PCL triblock copolymer and SIM loaded PCL-PEG-PCL triblock copolymer micelles

The preparation of PCL-PEG-PCL triblock copolymer and incorporated SIM into the micelles system was obtained from our previous study. Where PCL-PEG-PCL triblock copolymer is made by reacting 5 g of PEG and 10 g of ε-CL using Sn $(Oct)_2$ 0.5% w/w as a catalyst by the ring-opening polymerization method (ROP). While SIM was incorporated into the polymeric micelles by the solvent evaporation method (film formation), 1 ml of SIM stock solution in dichloromethane (100 mg/10 ml) was mixed with 50 mg of PCL-PEG-PCL triblock copolymer [15].

### Preparation of SIM stock solution

SIM was weighed as much as 10 mg, put into a 25 ml volumetric flask, and methanol was added to the mark and then homogenized to obtain a concentration of 400 ppm [16].

### Determination of the $\lambda_{max}$ of SIM

The 0.05 ml of the stock solution is pipetted, put into a 5 ml volumetric flask, distilled water is added to the limit mark and homogenized to obtain a solution with a concentration of 4 ppm, then the solution is measured using a UV-vis spectrophotometer over a 200-300 nm wavelength range. The $\lambda_{max}$ of SIM is indicated by the wavelength that gives the highest absorbance [16].

### Preparation of SIM calibration curve

The stock solution was pipetted as much as each 0.050, 0.075, 0.100, 0.125, 0.150, and 0.175 ml were put into a 5 ml volumetric flask, and then distilled water was added to the mark and homogenized to obtain a serial solution with a concentration of 4, 6, 8, 10, 12 and 14 ppm. The series solution was measured with a UV-vis spectrophotometer at the $\lambda_{max}$ of SIM [16].

### Solubility enhancement test of SIM in the PCL-PEG-PCL triblock copolymer micelles

SIM excess (10 mg) and SIM loaded into PCL-PEG-PCL triblock copolymer was dissolved in 10 ml of distilled water and shaken for 24 h

at 25±1 °C. After 24 h, the solution was filtered with a 0.45 µm membrane filter and measured using a UV-vis spectrophotometer at the $\lambda_{max}$ of SIM and the dissolved content was calculated using a calibration curve that had been prepared [20]. The instrument used was a UV-vis spectrophotometer (Thermo Scientific, Genesys 10S UV).

**Method validation**

**Linearity test**

The linearity test was carried out by analyzing the measurement results of the serial solution that had been made (4, 6, 8, 10, 12, and 14 ppm) then made a relationship between the absorbance and the concentration of the serial solution, a linear regression equation (y = ax+b) and correlation coefficient (R) was obtained [16].

**Determination of limit of detection and limit of quantification (LOD and LOQ)**

The LOD and LOQ was determined by measuring the absorbance of the serial solution on the calibration curve for 3 replications and then the standard deviation (SD) is determined [20].

LOD was calculated using the following equation:

$$LOD = \frac{3.3 \times SD}{Slope}$$

LOQ was calculated using the following equation:

$$LOQ = \frac{10 \times SD}{Slope}$$

**Accuracy and precision test**

The stock solution was pipetted as much as each 0.100, 0.125, and 0.150 ml, were put into a 5 ml volumetric flask containing 0.125 ml of PCL-PEG-PCL triblock copolymer solution in water, then distilled water was added to the mark, and then homogenized. The test solution was measured with a UV-vis spectrophotometer at the $\lambda_{max}$ of SIM. The accuracy test is assessed based on the % recovery, while the precision test is determined based on the relative standard deviation (RSD) value [16, 21].

**RESULTS AND DISCUSSION**

**Increased solubility of SIM in PCL-PEG-PCL triblock copolymers micelles**

Theoretically, SIM has a water solubility of 10 g/ml. The solubility of the modified SIM into the PCL-PEG-PCL triblock copolymer micelles was 89.49±5.75µg/ml, while the solubility of SIM without modification was 9.19±0.24µg/ml. These results indicate that there has been an increase in the solubility of SIM after being made into micelles [22]. The complete test results for increasing the solubility of SIM can be seen in table 1.

**Table 1: The results for increasing solubility of SIM in triblock copolymer**

| Sample | Concentration of SIM (µg/ml)* | Increased solubility |
|---|---|---|
| SIM without modification | 9.19±0.24 | - |
| SIM in micelles | 89.49±5.75 | 9.7 times |

*All values are expressed as mean of n=3±standard deviation (SD)

SIM is a drug that has low solubility in water. Drugs with low solubility in water will be difficult to absorb into the gastrointestinal tract where the main component is water so that it will cause low bioavailability. Therefore, one way that can be used to increase the bioavailability of a drug is to increase its solubility in water [1, 3]. A solubility enhancement test was carried out to examine the ability of PCL-PEG-PCL triblock copolymer to increase the solubility of SIM. A similar study was conducted by Alami-milani *et al.* using a hydrophobic drug model of dexamethasone, in that study, it was reported that the use of PCL-PEG-PCL triblock copolymers could increase the solubility of dexamethasone by 11.7 times (1.17 mg/ml) [23].

The PCL-PEG-PCL triblock copolymer was composed of PCL as a hydrophobic block and PEG as a hydrophilic block. In aqueous media, PCL-PEG-PCL triblock copolymer will spontaneously form micelles with the hydrophilic part on the outside and the hydrophobic part on the inside as the core [24, 25]. The ability to increase drug solubility by triblock micellar copolymers is influenced by the length of the hydrophobic block and the ratio of the constituent polymers. The longer the hydrophobic block that makes up the triblock copolymer, the greater the solubility of the drug because more hydrophobic drugs can be loaded into the micellar system [26].

**Analysis method validation**

Method validation was carried out on the parameters of the $\lambda_{max}$, correlation coefficient (R) of the obtained calibration curve, LOD, LOQ, accuracy, and precision. The complete validation test results for various parameters can be seen in table 2.

**Table 2: The summary of validation**

| Parameter | Result | |
|---|---|---|
| $\lambda_{max}$ | 239 nm | |
| Linearity | | |
| y = ax+b | Y = 0.0499x–0.0249 | |
| a: slope | | |
| b: intercept | | |
| Coeficient correlation (R) | 0.9994 | |
| LOD (µg/ml) | 0.33 | |
| LOQ (µg/ml) | 1.00 | |
| Accuracy (% recovery)* | 80% | 102.93±1.32 |
| | 100% | 100.78±0.40 |
| | 120% | 104.58±0.79 |
| Precision (% RSD) | Intra-day | 1.371 (8 µg/ml) |
| | | 0.418 (10 µg/ml) |
| | | 0.786 (12 µg/ml) |
| | Inter-day (10 µg/ml) | 0.418 (1st day) |
| | | 0.525 (2nd day) |

*All values are expressed as mean of n=3±standard deviation (SD)

**$\lambda_{max}$ of SIM measurement results**

The $\lambda_{max}$ is the wavelength that gives the maximum absorption of SIM. The determination of the $\lambda_{max}$ of SIM aims to provide maximum sensitivity of samples containing SIM, a calibration curve that is linear and produces fairly constant data if repeated measurements are made. Determination of the $\lambda_{max}$ was carried out at a concentration of 4 ppm using a Uv-Vis spectrophotometer, the fig. 1

showed the $\lambda_{max}$ of SIM was 239 nm, the results were not much different from the $\lambda_{max}$ of SIM in the literature, which was 238 nm. The $\lambda_{max}$ shift of the measurement results compared to the literature can be caused by several factors, such as differences in the source of materials and the tools used. However, the wavelength shift is not more than 3% of the $\lambda_{max}$ in the literature so it can be said that the results of the measurements carried out meet the requirements for use for analysis [16].

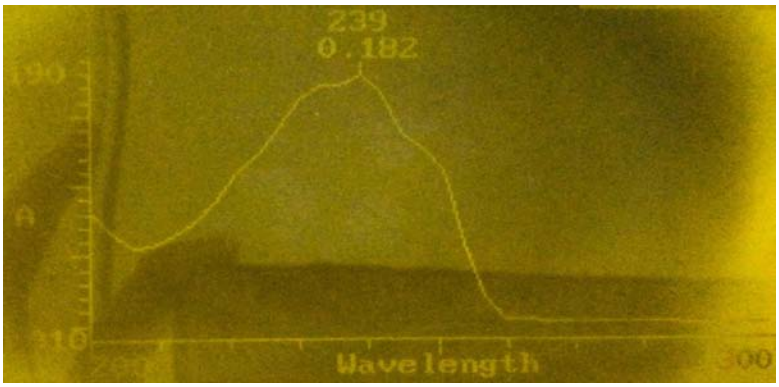


**Fig. 1: The $\lambda_{max}$ of SIM**

**The results of the calibration curve and linearity test**

The calibration curve was used to determine the linear regression equation that would be used to calculate SIM levels in the PCL-PEG-PCL triblock copolymer. The linear regression equation was obtained from the relationship between the concentration of the prepared SIM series solution and its absorbance measured at a wavelength of 239 nm which is the $\lambda_{max}$ of SIM used in this study, which was presented in table 3. SIM calibration curve graph can be seen in fig. 2, with intercept value =-0.0249 and slope value = 0.0499, so that the linear regression equation y = 0.0499x-0.0249 with R = 0.9994 is obtained. The linearity test can be determined based on the correlation coefficient (R) of the obtained linear regression equation, where the acceptance criteria of the linearity test are R 0.9994. When viewed from the R-value obtained in the test, it shows that the method used has good linearity [16, 17, 27].

**Accuracy and precision test results**

One of the fundamental requirements in the analysis is accuracy and precision. Accuracy indicates the closeness of the measurement results to the actual value which is expressed as % recovery, while precision indicates the degree of suitability of the test results as measured by the distribution of the results from the average when repeated measurements are made which will produce an average value that is very close to the true value. The measuring parameter to determine precision is the percent relative standard deviation (% RSD) [16, 17, 28].

Table 4 presents that the average % recovery obtained in determining the accuracy is 102.93±1.32%, 100.78±0.40%, and 104.58±0.79%. % recovery is acceptable because it is in the range of 80-110% [17, 21, 28].

**Table 3: The result of absorbance measured of the series solution**

| Concentration (ppm) | Absorbance* |
|---|---|
| 4 | 0.181±0,002 |
| 6 | 0.264±0,008 |
| 8 | 0.379±0,003 |
| 10 | 0.468±0,007 |
| 12 | 0.580±0,006 |
| 14 | 0.672±0,004 |

*All values are expressed as mean of n=3±standard deviation (SD)

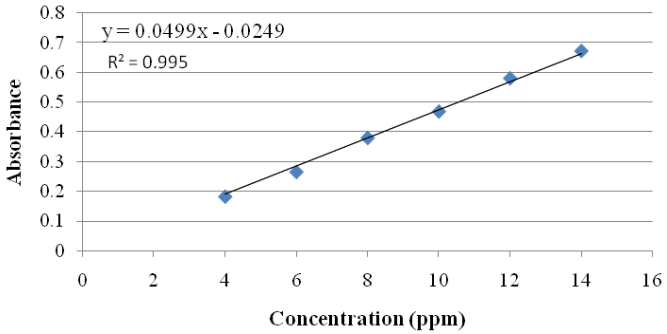


**Fig. 2: The graph of SIM calibration curve**

**Table 4: The results of accuracy test of SIM in PCL-PEG-PCL triblock copolymer micelles**

| Concentration (%) | Theoretical level (µg/ml) | Calculated level (µg/ml) | Recovery (%)* | % RSD |
|---|---|---|---|---|
| 80 | 8 | 8.234 | 102.93±1.32 | 1.269 |
| 100 | 10 | 10.078 | 100.78±0.40 | 0.398 |
| 120 | 12 | 12.549 | 104.58±0.79 | 0.755 |

*All values are expressed as mean of n=3±standard deviation (SD)

The precision test results are shown in table 5 and 6 show that the % RSD of the average SIM absorbance obtained was 1.371, 0.418, and 0.786 for the intra-day measurement, while 0.418 for the first day and 0.525 for the second day on the inter-day measurement with a concentration of 10 g/ml. The value of % RSD<2 indicates that the method shows good precision [13, 14].

**Table 5: The results of intra-day precision test of SIM in PCL-PEG-PCL triblock copolymer micelles**

| Concentration (µg/ml) | Absorbance* | % RSD |
|---|---|---|
| 8 | 0.386±0.005 | 1.371 |
| 10 | 0.478±0.002 | 0.418 |
| 12 | 0.601±0.005 | 0.786 |

*All values are expressed as mean of n=3±standard deviation (SD)

**Table 6: The results of inter-day precision test of SIM in PCL-PEG-PCL triblock copolymer micelles**

| Concentration (µg/ml) | Days- | Absorbance* | % RSD |
|---|---|---|---|
| 10 | 1st | 0.478±0.002 | 0.418 |
| | 2nd | 0.479±0.003 | 0.525 |

*All values are expressed as mean of n=3±standard deviation (SD)

## CONCLUSION

The solubility of SIM after being incorporated into PCL-PEG-PCL triblock copolymer micelles was successfully increased by 9.7 times compared to SIM without modification. The Uv-Vis spectrophotometer used to measure dissolved SIM levels has been successfully validated. The validation results show the $\lambda_{max}$ value of 239 nm, a linear calibration curve with an R-value of 0.9994, LOD and LOQ of 0.33 µg/ml and 1.00 µg/ml, accurate measurement with % recovery at concentrations of 80%, 100%, and 120% were 102.93±1,32%, 100.78±0.40% and 104.58±0.79% and also has a good precision value with RSD<2%.

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## AUTHORS CONTRIBUTIONS

All of the authors listed in this manuscript have contributed equally.

## CONFLICT OF INTERESTS

The author declares that there is no conflict of interest related to this report.

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