Development and Validation of High Performance Liquid Chromatography Method for Determination Atorvastatin in Tablet

A Yugatama¹, S Rohmani¹, A Dewangga¹
¹Department of Pharmacy, Faculty of Mathematics and Natural Sciences, Sebelas Maret University, Indonesia
E-mail: adiyugatama.apt@gmail.com

Abstract. Atorvastatin is the primary choice for dyslipidemia treatment. Due to patent expiration of atorvastatin, the pharmaceutical industry makes copy of the drug. Therefore, the development methods for tablet quality tests involving atorvastatin concentration on tablets needs to be performed. The purpose of this research was to develop and validate the simple atorvastatin tablet analytical method by HPLC. HPLC system used in this experiment consisted of column Cosmosil C18 (150 x 4.6 mm, 5 µm) as the stationary reverse phase chromatography, a mixture of methanol-water at pH 3 (80:20 v/v) as the mobile phase, flow rate of 1 mL/min, and UV detector at wavelength of 245 nm. Validation methods were including: selectivity, linearity, accuracy, precision, limit of detection (LOD), and limit of quantitation (LOQ). The results of this study indicate that the developed method had good validation including selectivity, linearity, accuracy, precision, LOD, and LOQ for analysis of atorvastatin tablet content. LOD and LOQ were 0.2 and 0.7 ng/mL, and the linearity range were 20 - 120 ng/mL.

1. Introduction
Atorvastatin is the statin derivatives that works with inhibiting HMG-CoA reductase in a competitive manner and the most commercially available drugs for the treatment of dyslipidemia [1]. Atorvastatin is the most potent drug to reduce LDL levels because its use with medium dose intensity (10 – 20 mg/day) evident effectively to reduces levels of LDL by 30 – 50% from previous, and with high dose intensity (40 – 80 mg/day) evident capable of reduces LDL levels of > 50% from previous [2]. Due to patent expiration of Lipitor® which is a drug patent atorvastatin in 2011 [3], the pharmaceutical industry makes copy of the drug. Therefore, tablet quality tests involving atorvastatin levels on tablets needs to be performed. To achieve it needs to be developed the method of analysis atorvastatin levels in tablet.

The development method of analysis levels of an active substance in tablet with high performance liquid chromatography (HPLC) has become the common method because it is easy enough to operated and time of analysis is relatively short. In previous studies a mobile phase used was mixture of a solvent acetonitril-buffer ammonium acetate (60:40 v/v) [4]. But in that method, the use of buffer solution as mobile phase would be easy clogging the columns and longer laundering. The use of mixture of methanol and water for mobile phase was more efficient than use of buffer because its not clogging the columns, not require mobile phase preparation, and the selectivity of methanol is better than acetonitril [5]. Therefore, it is necessary to develop of atorvastatin analysis method by high performance liquid chromatography (HPLC) with a mixture of methanol and water as
mobile phase that meets the validation parameters including: selectivity, accuracy, precision, linearity, LOD (Limit of Detection), and LOQ (Limit of Quantitation).

The purpose of this study was to obtain a simple method for analyzing the content of atorvastatin tablets by high performance liquid chromatography (HPLC) that meets the validation parameters including selectivity, linearity, accuracy, precision, LOD (Limit of Detection), and LOQ (Limit of Quantitation).

2. Experimental

2.1. Instrument

The instrument used were High Performance Liquid Chromatography (Lab Alliance) featuring Rheodyne sample injectors (20 μL sample loops), UV SCL-10A VP detector (Lab Alliance) and LC-10AT VP pump (Lab Alliance), Cosmosil C18 Column (150 x 4.6 mm, 5 μm), UV-Vis spectrophotometer (Shimadzu AA 6650), analytical balance scale (Ohaus PA413), glass tools (Pyrex), and other supporting tools.

2.2. Material

The materials used were atorvastatin standard (PT Kalbe Farma, Indonesia), methanol for HPLC (Merck), and aquabidest (PT Ikapharmindo).

2.3. Stock solution Preparation

Atorvastatin stock solution made with a concentration of 1 mg/mL, using 100 mg of standard atorvastatin dissolved in methanol up to 100 mL.

2.4. Determination of the maximum standard atorvastatin wavelength (scanning λ) in the mobile phase

Stock solution of standard atorvastatin was created in concentration of 100 μg / mL. Scanning of maximum wavelength was performed by UV-Vis spectrophotometer at 200-400 nm.

2.5. Optimization of mobile phase composition and flow rate on HPLC systems

A 20 μL standard atorvastatin solution with a concentration of 100 ng/mL was injected into the HPLC system at maximum wavelength and flow rate of 1 mL/min, where variations in mobile phase composition and mobile phase flow rate were performed. Furthermore, the composition and flow rate of the mobile phase was determined by the best separation performance, based on retention time (Rt), resolution (Rs), tailing factor (TF), and theoretical plate (N). Required separation requirements were retention time < 10 min, Rs > 2, TF ≤ 2, and N > 2000 [5].

2.6. System Suitability Test

System suitability test was done by making concentration of standard solution of 120 ng/mL. A 20 μL standard 120 ng / mL atorvastatin solution was injected into the HPLC system. Based on chromatogram obtained, retention time (Rt), peak area, and peak height were set. The system suitability test was meet the qualification if the CV value was 2.0% for the area and peak height, and ≤ 1.0% for retention time, TF ≤ 2, Rs ≥ 2, and N > 2000 [5].

2.7. Validation of Analysis Methods

Validation methods including (i) selectivity testing by comparing standard chromatograms of atorvastatin, atorvastatin tablets, and mobile phase solvents, (ii) determination of linearity by making series of concentrations of 20 - 120 ng / mL, (iii) accuracy and precision testing, (iv) determination of LOD and LOQ.
2.8. Analysis of samples of atorvastatin tablets
Each atorvastatin tablet were weighed and crushed, then dissolved in 10 mL of methanol, homogenized, and was filtered. The solution was diluted in mobile phase by 20,000 times. A 20 μL was injected in the HPLC system.

3. Result and Discussion

3.1. Determination of the maximum standard atorvastatin wavelength (scanning λ)
The results of maximum wavelength scanning of atorvastatin in a methanol-water pH 3 (80:20 v/v) mobile phase with a concentration of 100 μg/mL using a UV-Vis spectrophotometer at a wavelength of 200-400 nm showed that atorvastatin had maximum absorption at 245 nm. Scanning results can be seen in Figure 1.

3.2. Optimization of mobile phase composition and flow rate on HPLC systems
From the optimization result of mobile phase composition in table 1, the 80:20 ratio was chosen because it yielded the best resolution and tailing factor values with a reasonably fast retention time compared to other mobile phase compositions. In addition, the result of N value was also qualified (N > 2000) [5]. Furthermore, by the result of the mobile phase flow rate optimization in table 2, the chosen flow rate was 1 mL/min as it yields optimum resolution, tailing factor, N value, peak area and retention time.

3.3. System Suitability Test
The result of the system suitability test seen in table 3, indicates that the HPLC system used for analysis of atorvastatin levels in tablets meets the requirements of the system suitability test.

3.4. Validation of Analysis Methods
In the selectivity determination, it was resulted a chromatogram with a peak of atorvastatin standard and sample at 3.9 min retention time, and there was no peak seen in mobile phase solvent chromatogram. This showed that the method used was selective for atorvastatin analysis. In the linearity determination, 5 series of standard atorvastatin concentrations of 20 – 120 ng/mL obtained r² more than 0.997 so it meets the requirements of linearity [6]. In the determination of accuracy and precision result in table 4, it was indicating that the recovery value and CV has fulfilled the requirements of 80 - 110% recovery value and < 7.3% CV value [7]. In the LOD determination, was used visual instrumental method with the calculation of signal to noise ratio [8]. The results obtained that LOD and LOQ values were 0.2 ng/mL and 0.7 ng/mL.

3.5. Tablet Uniformity Test
The result of uniformity test of tablet sample in Table 5 showed that the atorvastatin concentration in tablet sample was meet the requirements of the content not less than 90.0% and not more than 110.0% than indicated on the label [3].
Figure 1. UV Spectrum of Atorvastatin in the Mobile Phase

Table 1. Optimization Result of Mobile Phase Composition

| Parameter                  | Mobile Phase Composition (Methanol-Water pH 3) | Requirement (Snyder et al., 2010) |
|---------------------------|-----------------------------------------------|-----------------------------------|
|                          | 70:30 | 75:25 | 80:20 | 85:15 | 90:10 |                  |
| Resolution (Rs)           | 1.54  | 0.83  | 3.15  | 1.06  | 3.00  | > 2               |
| Rt (min)                  | 6.24  | 4.98  | 3.91  | 3.25  | 2.72  | ≤ 10              |
| Tailing Factor (TF)       | 1.6   | 0.89  | 0.9   | 0.75  | 1.2   | < 2               |
| Theoretical Plate Number (N) | 5784  | 5377  | 5184  | 4210  | 5980  | > 2000            |

Table 2. Optimization Result of Flow Rate

| Parameter                  | Flow Rate (mL/min) | Requirement (Snyder et al., 2010) |
|---------------------------|-------------------|-----------------------------------|
|                          | 0.9   | 1.0   | 1.1   |                  |
| Resolution (Rs)           | 3.00  | 3.15  | 2.63  | > 2               |
| Rt (min)                  | 4.49  | 3.91  | 3.61  | ≤ 10              |
| Tailing Factor (TF)       | 1.12  | 0.90  | 0.75  | < 2               |
| Theoretical Plate Number (N) | 5367  | 5184  | 5184  | > 2000            |

Table 3. System Suitability Test Result

| Parameter                  | Average | CV (%) | Requirement (Snyder et al., 2010) |
|---------------------------|---------|--------|-----------------------------------|
| Retention time (Rt)       | 3.96    | 0.17   | CV ≤ 1.0%                         |
| Peak area                 | 2726465 | 0.77   | CV ≤ 2.0%                         |
| Peak height               | 362225  | 0.51   | CV ≤ 2.0%                         |
| Resolution (Rs)           | 2.45    | 2.02   | RS > 2                            |
| Tailing Factor (TF)       | 0.88    | 1.67   | TF ≤ 2                            |
| Theoretical Plate Number (N) | 6055  | 0.07   | N > 2000                          |
Figure 2. Determination Result of Linearity

$y = 10.742.61x + 1.356.494,32$

$R^2 = 0.999$

### Table 4. Accuracy and Precision Result

| Atorvastatin Concentration are Known (ng/mL) | Measured Atorvastatin Concentration (ng/mL) | Recovery (%) | CV (%) |
|---------------------------------------------|--------------------------------------------|--------------|--------|
| 20                                          | 20.22                                      | 101.15       |        |
|                                             | 20.66                                      | 103.32       |        |
|                                             | 19.02                                      | 95.09        | 0.64   |
|                                             | 20.96                                      | 104.85       |        |
|                                             | 18.92                                      | 94.62        |        |
| 40                                          | 43.59                                      | 108.99       |        |
|                                             | 39.66                                      | 99.16        |        |
|                                             | 38.49                                      | 96.22        | 1.38   |
|                                             | 37.53                                      | 93.84        |        |
|                                             | 39.72                                      | 99.30        |        |
| 60                                          | 58.28                                      | 97.14        |        |
|                                             | 56.15                                      | 93.59        |        |
|                                             | 56.12                                      | 93.53        | 1.35   |
|                                             | 56.73                                      | 94.55        |        |
|                                             | 62.02                                      | 103.37       |        |
| 80                                          | 81.58                                      | 101.98       |        |
|                                             | 81.31                                      | 101.63       |        |
|                                             | 73.84                                      | 92.31        | 1.73   |
|                                             | 75.04                                      | 93.79        |        |
|                                             | 78.40                                      | 98.01        |        |
| 120                                         | 111.69                                     | 93.08        |        |
|                                             | 129.53                                     | 107.95       |        |
|                                             | 119.80                                     | 99.86        | 2.82   |
|                                             | 123.18                                     | 102.65       |        |
|                                             | 115.25                                     | 96.05        |        |
Table 5. Result of Uniformity Test

| Tablet | Concentration (%) | SD  | CV  |
|--------|------------------|-----|-----|
| 1      | 105.09           |     |     |
| 2      | 100.52           |     |     |
| 3      | 105.82           |     |     |
| 4      | 102.45           |     |     |
| 5      | 102.55           |     | 1.79| 1.75|
| 6      | 101.93           |     |     |
| 7      | 103.60           |     |     |
| 8      | 100.34           |     |     |
| 9      | 102.09           |     |     |
| 10     | 101.51           |     |     |

4. Conclusion
1. Simple method of analysis by HPLC resulting optimum conditions for determination of atorvastatin tablet content were using a mixture of methanol-water pH 3 (80:20 v/v) as mobile phase; a C18 column as stationary phase, a flow rate of 1 mL / min and detected with a 245 nm UV detector.

2. The simple analytical methods developed have met the validation requirements of the analysis method including selectivity; linearity in the range 20 - 120 ng/mL with $r^2 = 0.999$; accuracy; precision; Limit of Detection (LOD) of 0.2 ng/mL; and Limit of Quantitation (LOQ) of 0.7 ng/mL.

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