Original Article

Method Of The Development And Validation For The Determination Of Atorvastatin In Both Bulk And Commercially Pharmaceutical Products

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Abstract

A new method was developed and validated utilizing Nanodrop 2000 spectrophotometric for the measurement of Atorvastatin calcium trihydrate (ACT) in both bulk and tablet dosage forms. Atorvastatin's highest absorption was observed at 243 nm using Methanol as a solvent. The developed method was discovered to be linear (R2 = 0.9997) between 5 and 25 µg/mL in terms of concentration. Acceptable RSD% values (less than 0.5%) are provided by the precision and accuracy research. The results showed that the LOD and LOQ were, respectively, 0.446 and 1.488 µg/mL. The essay analysis revealed excellent recovery at around 98% for drug products in commercial tablets. It can be concluded that the present method is acceptable according to the International Conference on Harmonization (ICH) guidelines for drugs.

KEYWORDS: UV nanodrop 2000c, Development, Validation, Quality control, Atorvastatin.

1 INTRODUCTION

Hyperlipidemia is a known risk factor for the development of coronary artery disease and the progression of atherosclerotic lesions. Antihyperlipidemic Dietary therapy with lipid-lowering drugs is critical for hyperlipidemia management [1].

Atorvastatin calcium trihydrate (ACT) is a hypolipidaemic belonging to lipid-lowering drugs that reduce the level of “bad” cholesterol LDL Low-Density Lipoproteins and TG triglycerides in the blood also help to increase the level of the “good cholesterol” (HDL or High-Density Lipoproteins). It is an HMG-CoA reductase inhibitor. The IUPAC name of Atorvastatin calcium trihydrate (ACT) (brand name Lipitor®) is Calcium (3R,5R)-7-(2-(4-fluorophenyl)-5-isopropyl-3-phenyl-4-(phenylcarbamoyl)-1H-pyrrol-1-yl)-3,5-dihydroxyheptanoate trihydrate. The molecular formula of Atorvastatin calcium trihydrate (ACT) is C66H74CaF2N4O13 its molecular weight is 1209,4. The structural formula of ACT is shown in Figure1[2]. In evaluating and reviewing the literatures, many analytical techniques were created and approved for the detection of ACT alone or in combination with other medications, including spectrophotometry[3–5], high-performance liquid chromatography (HPLC)[6–9], Fourier Transform Infra-Red (FTIR)[10], thin layer chromatography (TLC) [11].

The NanoDrop 2000 technology is based on an innovative sample retention system that uses surface tension to hold and measure microvolume samples between two optical pedestals without the use of cuvettes or capillaries. Metal nanoparticle (NP) colloids with diameters (d) ranging from 1 to 100 nm exhibit a distinct optical absorption that is related to the oscillation of surface electrons. The surface plasmon resonance (SPR) property of the NP is affected by its size and shape, as well as the surrounding medium. The NanoDrop 2000 is ideally suited for measuring Kinetic methods, Custom methods, Purified protein analysis (A280), General UV-Vis spectrophotometry, etc [12], [13], [14]

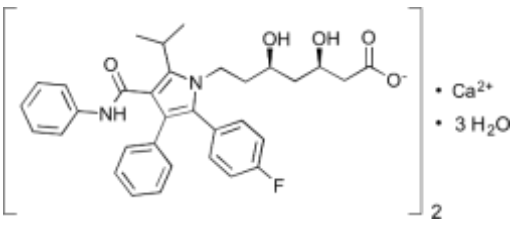

Figure 1. The Chemical structure of Atorvastatin calcium.

2 Materials and Methods

2.1 Chemicals

A Pharmaceutically active ingredient of Atorvastatin calcium trihydrate (ACT) (99 % purity) was acquired from A company in Kurdistan region of Iraq named Awa Medica while the commercial tablets of Atorvastatin Awa, Liponeer, Vastor, Ateroz, Avast, and Atorvast (from different companies) were randomly purchased from local Pharmacies. Acetonitrile, ethanol, water, and methanol (all from Merck, Germany) were HPLC grade solvents.

2.2 Instrumentation

A Thermo Scientific nanodrop spectrophotometer (2000C Micro volume) was used for the spectrophotometric analysis. Voyager® was the analytical balance. Elmasonic P (100W, 80 kHz) manufactured the oven, and Lab Tech. manufactured the water bath shaker (LVO-2030).

2.3 Preparation of working solutions and stock

To prepare a stock standard solution containing 1000 µg/mL of pure ACT, accurate weighed 0.25 g of the pure drug, dissolved in methanol solvent, and transferred to a 250 mL volumetric flask. The volume was then made up to the mark with methanol solvent. To make various concentrations of working solutions, we used this stock standard solution, which maintained in a refrigerator at temperature 4 °C.

2.4 Method Optimization

Using methanol as a blank, the maximum adsorption wavelength (max) of ACT solution 50 µg/mL was determined utilizing a nanodrop spectrophotometer between the wavelengths of 200 and 700 nm. A preliminary analysis of ACT's solubility was also carried out using the solvents acetonitrile, water, and ethanol.

2.5 Method Validation

The nanodrop spectrophotometric method was validated in terms of system, linearity, precision, LOD, LOQ, accuracy, specificity, and robustness in accordance with the International Conference on Harmonization (ICH) guidelines.

2.6 Formulation stock solution preparation

Ten tablets (1.0 g) of each commercial product (each tablet contains 20 mg of pure ACT) were precisely weighed and powdered. In a 150 mL conical flask, 0.5g of tablet powder, equivalent to 0.1 g of ACT, was placed, 50 mL of the solvent was added, and the flask was sonicated for 10 minutes. The solution was filtered in a 100 mL volumetric flask using Whitman filter paper (0.45 µm), and the volume was filled with methanol solvent to form a stock solution containing 1000 µg/mL of ACT for each of the Six drug products.

3 Result and discussion

3.1 Method development

Analytical technique development and validation strives to validate an appropriate method for analyzing a specific analyte with specific, precise, and accurate results. A wide range of processes need to be tested because the main goal of that is to improve the circumstances and parameters used in the development and validation methods.

3.1.1 The λmax determination and scaning:

As shown in Figure 2, the spectrum of ACT showed maximum absorbance at 243 nm. All subsequent research measures were conducted at this wavelength.

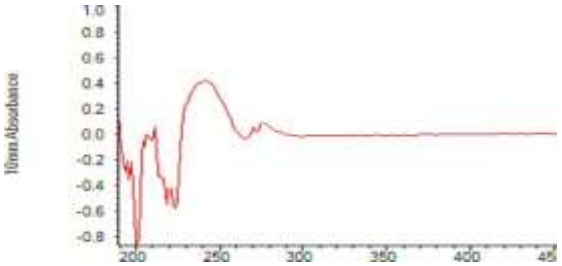

Figure 2: UV-Visible spectrum of ACT 243 nm

3.1.2 Type of solvent:

The influences of several solvents, such as water, methanol, ethanol, and acetonitrile were investigated. 20 mL of solvents were used to test the solubility of pure ACT. the pure drug was soluble more easily in methanol rather than in acetonitrile, ethanol, and water. The absorbance was measured and discovered that pure drug in methanol had the maximum absorbance (0.74) at 243 nm compared to ethanol (0.66), water (0.32), and acetonitrile (0.534). Therefore, according to the highest absorbance, methanol was used in this research as a solvent.

3.2 Method Validation

Method validation is a data gathering and analysis procedure that establishes exact proof that an analytical method is capable of providing high-quality results. In accordance with the ICH guidelines developed method was validated for the precision, linearity, accuracy, LOD, LOQ, specificity, and robustness.

3.2.1 Linearity:

Linearity, typically referred to as the confidence limit around the regression line's slope, is the technique's capacity to compile test outcomes that are proportionate to the amount of analyte in the sample (in a certain range) [15]. The method's linearity was estimated by examination and analyzing of five concentrations within range of 5-25 µg/mL. As shown in Figure 3, The regression equations for ACT were found to be Y = 0.0374X + 0.0002 with correlation coefficients (R2) 0.9997.

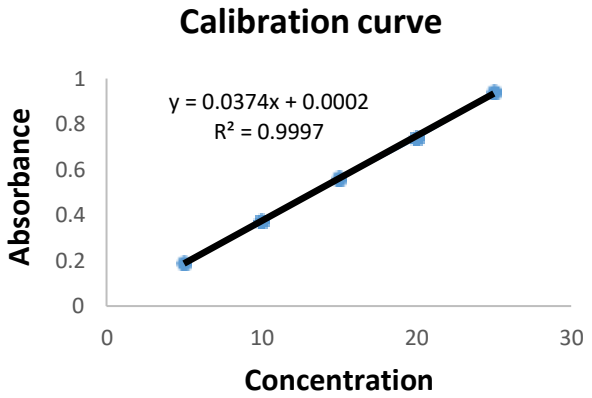

Figure 3: Calibration curve 5-25 µg/mL of ACT.

3.2.2 Precision:

Precision is described as how closely a set of measurements obtained through multiple sampling of the same homogenous sample agree with one another [16]. Through repeated injection, intra-day, and inter-day studies of ACT solution containing 5 µg/mL, the precision was assessed. Table 1 displays RSD% values that are less than 0.5%, which, by ICH standards, indicate good precision.

Table 1: Evaluation of precision study.

	Repeatability			Intra-day			Interday		
No. of sample	5	10	15	5	10	15	5	10	15
1	0.189	0.373	0.562	0.189	0.373	0.562	0.19	0.374	0.563
2	0.189	0.374	0.561	0.188	0.374	0.561	0.189	0.375	0.563
3	0.188	0.373	0.562	0.189	0.373	0.56	0.19	0.374	0.561
Mean (%)	0.188	0.373	0.562	0.19	0.37	0.56	0.19	0.37	0.56
SD	0.0009	0.0004	0.0007	0.0006	0.0006	0.0010	0.00	0.00	0.00
% RSD	0.47	0.12	0.13	0.31	0.15	0.18	0.30	0.15	0.21

3.2.3 LOD and LOQ:

The limit of detection of the procedure is the smallest quantity of an analyte that can be identified in a sample but not necessarily quantitated as a precise value. is the smallest amount of analyte in a sample that can be quantified with sufficient accuracy and precision[17]. These formulas can be used to determine LOD and LOQ:

3.3 * σ / S = LOD (1)

10 * σ / S = LOQ (2)

Where S is the slope of the calibration curve and σ is the standard deviation of the response, Table 2 displays LOD and LOQ values at the lowest levels of ACT.

Table 2 shows the LOD and LOQ values obtained from the calibration curve of ACT concentrations ranging from 2 to 10 µg/mL.

Parameters used	**Values**
The range of Linearity (µg/mL)	5-25
The equation of Regression	Y = 0.0374X + 0.0002
R^2	0.9997
The Slope	0.0374
The Intercept	0.0002
Standard deviation	0.0056
Limit of detection µg/mL	0.446
Limit of Quantitation µg/mL	1.488

3.2.4 Accuracy:

In a recovery investigation, samples from three replicates were tested at three concentration levels of the proposed approach to determine its validity and accuracy 50%, 100%, and 150% of ACT pure drug. The recovery results were shown in Table 3.

Table 3: Evaluation of accuracy study of pure ACT drug.

Level of Recovery (%)	Conc. added	Amount of Pure Drug Abs	Conc. Obtained	% Recovery	Statistical Analysis		
					Mean (%)	**SD**	**% RSD**

50%	5	0.189	5.1	102.16	101.80	0.31	0.31
	5	0.188	5.1	101.62			
	5	0.188	5.1	101.62			
100%	10	0.373	10.1	100.81	101.08	0.27	0.27
	10	0.375	10.1	101.35			
	10	0.374	10.1	101.08			
150%	15	0.561	15.2	101.08	101.02	0.10	0.10
	15	0.561	15.2	101.08			
	15	0.56	15.1	100.90			

3.2.5 Specificity:

The specificity of the current method was assessed by adding (starch, lactose, and magnesium) into the standard ACT solution and determining the percentage of recovery across three replications; the result was determined to be 100.05% with an RSD% of 0.25. The results showed that there was no excipient interference in the analysis of standard ACT.

3.2.6 Robustness:

Robustness is a measure used to assess a method's resistance to minor changes in its input parameters[16]. It was investigated how the wavelength change from 241 to 245 affected absorbance. A standard ACT solution (5 µg/mL) was prepared, and examined using a different wavelength. As shown in the Table 4, the RSD% for the measurement of absorbance was observed to be less than 1.5%.

Table 4: Robustness study of ACT at different wavelength range.

No. of sample	Absorbance at 241nm	Absorbance at 242nm	Absorbance at 243nm	Absorbance at 244nm	Absorbance at 245nm
1	0.19	0.185	0.189	0.187	0.187
2	0.19	0.189	0.19	0.185	0.188
3	0.188	0.189	0.191	0.191	0.19
4	0.189	0.191	0.189	0.189	0.189
5	0.187	0.189	0.19	0.189	0.189
Mean	0.1888	0.1886	0.1898	0.1882	0.1886
SD	0.0013	0.0022	0.0008	0.0023	0.0011
% RSD	0.6906	1.1617	0.4408	1.2117	0.6045

3.2.7 Essay of drug products:

To ensure the accuracy of pure sample, the recovery and RSD% experiments were performed using a known concentration of the commercial products of ACT. The percentage recovered was depicted in Table 5.

Table 5: Recovery and RSD% of ACT products.

Tablet formulation	Conc. Added	Conc. Founded	Recovery %	RSD%
Atrovastatine pure drug	20µg/ml	20.06	100.31	0.07
Atrovastatine Awa	20µg/ml	19.75	98.93	0.13
Liponeer	20µg/ml	19.77	98.89	0.20
Ateroz	20µg/ml	19.82	99.11	0.07
Avast	20µg/ml	20	100.04	0.050
Atorvast	20µg/ml	20.01	100.09	0.27

Vastor	20µg/ml	20.04	100.22	0.07

4 Conclusion

According to ICH recommendations, the Nanodrop 2000 UV-spectrophotometric method was developed and validated for the detection of Atorvastatin calcium trihydrate (ACT) in tablet dose formulation. The results demonstrated that the method was satisfactory, as each of the measured parameters met the acceptance criteria recommended by the ICH. The developed method was found to be rapid, linear, accurate, precise, robust, specified, and, economic. The results demonstrated that the method was adequate, as each of the measured parameters met the acceptance criteria recommended by the ICH. Conclusively, the developed analytical method can be used for quantitative analysis of Atorvastatin calcium trihydrate in both bulk and tablet dosage forms.

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