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UV and First Derivative Spectrophotometric Methods for the Estimation of Atorvastatin in Pharmaceutical Preparations

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ABSTRACT

Objective: In this study, new, rapid UV spectrophotometry (UV) and first-order derivative spectrophotometry (^{1}D) methods were developed for the determination of atorvastatin in pure and tablets. **Methods**: The solvent system and wavelength of detection were optimized in order to maximize the sensitivity of the proposed methods. Parameters such as linearity, precision, accuracy, specificity, stability, limit of detection and limit of quantification were studied according to the International Conference on Harmonization Guidelines.**Results**: Calibration curve was linear between the concentration range of 5-20 µg ml^{-1}. Within- and between-day precision values for atorvastatin were less than 4.57%, and accuracy (relative error) was better than 3.17%. The mean recovery value of atorvastatin was 100.1% for pharmaceutical preparations. **Conclusion**: The developed methods were successfully applied to tablet formulations and the results were compared statistically with each other.

Keywords: *Atorvastatin; First-order derivative spectrophotometry; UV spectrophotometry; Validation*

INTRODUCTION

Atorvastatin is a drug of statins class. It is used in elevated blood cholesterol levels. It is chemically [R-(R*,R*)]-2-(4-fluorophenyl)-b,d-dihydroxy-5-(1-methylethyl)-3-pheny1-4 [(phenyl amino) carbonyl]-1H-pyrrole-1-heptanoic acid, calcium salt (2:1) trihydrate **(Figure 1).** Its molecular formula is $C_{66}H_{68}CaF_2N_4O_{10}$ and its molecular weight is 1209.42. It is a synthetic cholesterol lowering agent[1,2].

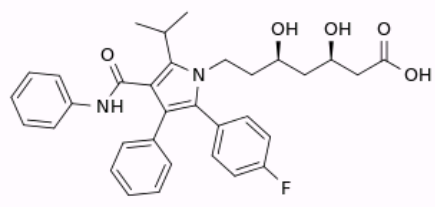

Figure 1 Chemical structure of atorvastatin

Using of atorvastatin leads to reducing the total cholesterol, low-density lipoprotein cholesterol[3], apo-B[4], triglycerides levels[5], and CRP[6] as well as increasing HDL levels. This drug also stabilizes plaque and prevents risk of strokes, heart attack or other heart complications through anti-inflammatory and other mechanisms.

Several methods have been reported for the determination of atorvastatin in pharmaceutical formations and in biological fluids including reversed-phase high performance liquid chromatography[7,8], liquid chromatography tandem mass spectrometry[9], high performance liquid chromatography[10] and spectrophotometry[11,12]. Different methods are reported on the electro oxidation and determination of atorvastatin, including adsorptive stripping voltammetry using glassy carbon electrode[13,14], cyclic and differential pulse voltammetry at a carbon paste electrode in the presence of an enhancing agent,

cetyltrimethylammoniumbromide[1] and differential pulse voltammetry using boron-doped diamond electrode and glassy carbon electrode[15].

However, to our knowledge, there is no individual first-order derivative spectrophotometric method for the determination of atorvastatin in pharmaceutical preparations in literature. Derivative spectrophotometry is an analytical technique of great utility for extracting both qualitative and quantitative information from spectra composed of unresolved bands, and for eliminating the effect of baseline shifts and baseline tilts. It consists of calculating and plotting one of the mathematical derivatives of a spectral curve[16]. Last year, this technique rapidly gained ground in application in the analysis of pharmaceutical preparations.

We wanted to develop two new spectrophotometric methods for the determination of atorvastatin in pharmaceutical preparations without the necessity of sample pre-treatment. After developing spectrophotometric methods were also carried out and all optimization parameters were also considered. Also, the developed methods were applied to commercial preparations (Ator, Cholvast and Lipitor) as tablet. The results obtained were statistically compared.

MATERIALS AND METHODS

Chemicals

Atorvastatin was purchased from Fluka (Buchs, Switzerland). Ator, Cholvast and Lipitor tablets were obtained from pharmacy (Erzurum, Turkey).

Equipment

A Thermospectronic double-beam UV-Visible spectrophotometer (HEλIOSβ) with a fixed slid width 2 nm and with a data processing system was used. UV and ^{1}D spectra (N=6,Δλ=4.0 nm) of standard and sample solutions were recorded in 1 cm quartz cells between wavelength ranges of 220-320 nm at scan speed of 600 nm min^{-1} and derivation interval (Δλ) 21.0 nm.

Preparation of standard curve for UV and ^{1}D methods

Stock solution of atorvastatin (100 µg ml^{-1}) was prepared by dissolving 10 mg atorvastatin in 100 mL of methanol. Working solutions (WS) containing 5, 7.5, 10, 12.5, 15, 17.5 and 20 µg ml^{-1} of atorvastatin were daily prepared by diluting the stock solution with a constant volume of methanol. The WS were prepared daily in analysis. Solutions were transferred to quartz cells for analysis. Stock solution was stored at -20 ºC in glass flask and brought to room temperature before use. Quality control (QC) samples were prepared by adding aliquots of standard solution of atorvastatin to final concentrations of 6, 13 and 19 µg ml^{-1}.

Data analysis

All statistical calculations were performed with the Statistical Product and Service Solutions (SPSS) for Windows, version 10.0. Correlations were considered statistically significant if calculated P values were 0.05 or less.

RESULTS AND DISCUSSION

Optimization of spectrophotometric conditions

To develop a sensitive UV and ^{1}D spectrophotometric method, the experimental conditions such as the solvent, the degree of derivation, the wavelength range and smoothing were optimized. Optimum results were obtained by measuring the wavelength range 220-320 nm through using high smoothing (Δλ = 21.0 nm) for UV and first-order derivative spectrophotometry. In this assay, various solvent systems such as water, methanol, ethanol and acetonitrile were tried either individually or in combinations of different proportions. The final decision of using methanol was based on sensitivity, interference, and easy preparation, suitability for drug, content estimation and cost, respectively. Methanol was used in this study because it has no toxicity. **Figures 2** and **3** present the overlay of UV and ^{1}D spectra of atorvastatin in the concentration of 5-20 µg ml^{-1}in methanol, respectively. Each spectrum can be used for the determination of this drug.

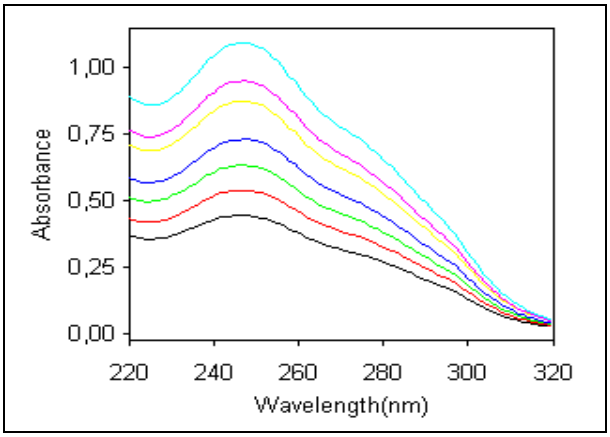

Figure 2. Spectra obtained from UV method (5, 7.5, 10, 12.5, 15, 17.5 and 20 µg ml^{-1})

The maximum peak at 247 nm was observed in UV spectra of atorvastatin. The maximum peak at 237 nm and a minimum peak at 261 nm were observed in the ^{1}D spectra of atorvastatin **(Figure 3).**

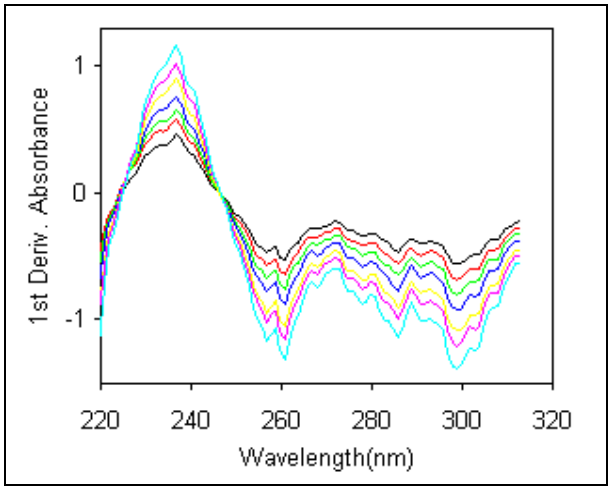

Figure 3. Spectra obtained from first-order derivative method (5, 7.5, 10, 12.5, 15, 17.5 and 20 µg ml^{-1})

Method validation

Linearity of calibration curves

In the UV and ^{1}D spectrophotometry method, the working solutions were scanned at 220-320 nm against a similarly prepared blank. The 247 nm wavelength for UV and the 237 and 261 nm wavelengths for ^{1}D method were used for calibration curves. Six-level calibration series with six analyses at each concentration level were measured. The standard calibration curves of atorvastatin in all wavelengths were constructed by plotting the A(Absorbance values for UV spectrophotometric method) and the $dA/d\lambda$ (first derivative values for first-order derivative spectrophotometric method) versus atorvastatin concentrations.

For all calibration curves, a good linearity within the concentration range of 5-20 µg ml^{-1} was shown for UV and ^{1}D methods. The regression equations were obtained by the least-square regression method. The calibration curves, regression equations and correlation coefficients found for UV and ^{1}D method were given in **Table 1.**

The correlation coefficient of standard calibration curves at 247 nm for UV spectrophotometric method and at 237 and 261 nm wavelength for ^{1}D method of atorvastatin in methanol was higher.

Sensitivity

Spectrophotometrically, the limit of detection (LOD) and the limit of quantification (LOQ) were determined by an empirical method that analyzed a series of standard solutions which were containing decreasing amounts of atorvastatin. LOQ was defined as the lowest concentration of measured value (RSD< 10%) and accuracy (80-120 %)] of standard solutions in calibration curves. LOD determined (RSD< 10%) as the lowest concentration of analyte which was distinguished from the blank with reasonable confidence was also calculated. LOQ and LOD values for both methods of standard atorvastatin solutions were found as 3.0 µg ml^{-1} and 1.0 µg ml^{-1}, respectively. All the RSD values were found lower than 10%.

Repeatability

Repeatability is given as within-day and between-day precision and accuracy where it was evaluated via analysis of three different concentrations of atorvastatin on six different days. Six replicate determinations at three different concentrations (6, 13 and 19 µg ml^{-1}) in 247 nm wavelength (for UV method) and, 237 and 261 nm wavelengths (for ^{1}D spectrophotometry method) were carried out to test the precision of these methods. The precision of the methods were given as the relative standard deviation (RSD=100 x Standard deviation/Mean) and the accuracy of these methods were given as the percent of mean deviation from known concentration [relative error; (concentration found-known concentration) x 100 / known concentration]. All samples were freshly prepared. For UV and ^{1}D spectrophotometry methods, the within-day precision showed that acceptable RSD% values which were <1.17% and <4.01% (n=6), respectively, and the between-day precision (intermediate precision) showed that acceptable RSD% values which were <1.35% and <4.57% (n=6), respectively **(Table 2)**.

Accuracy of UV and ^{1}D spectrophotometry methods showed that acceptable relative error values which were <0.77% and <3.17% (n=6), respectively **(Table 2)**.

Analytical recovery

To check accuracy of the proposed spectrophotometric methods, the standard addition technique was applied. The three different concentrations (2.5, 7.5 and 12.5 µg ml^{-1}) of pure sample solution were added to 5 µg ml^{-1}concentration of tablet solution and assayed. The analytical recovery of the added standard to the assay samples was calculated from followed equation:

$$\text{Recovery \%} = [(C_t - C_u) / C_a] \times 100$$

Where C_t is total concentration of the analyte determined; C_u is the concentration of the analyte present in the formulation; and C_a is the concentration of the pure analyte added to the formulation. The results of analysis of the commercial tablet and the recovery study were given in **Table 3.**

The average percent recoveries obtained were quantitatively as 100.3% for UV method and 99.8% for ^{1}D method, indicating good accuracy of the methods. No interference from the common excipients was observed.

Table 1. Results of regression analysis of proposed methods

Methods	Range (μg ml^{-1})	λ (nm)	LR[a]	Sa	Sb	R	LOD (μg ml^{-1})	LOQ (μg ml^{-1})
UV	5-20	247	y=0.0425x-0.0184	0.0058	0.0004	0.9986	1.00	3.00
^{1}D	5-20	237	y=0.0513x-0.0119	0.0023	3.42^{E-5}	0.9987	1.00	3.00
	5-20	261	y=0.0457x+0.0471	0.0041	4.37^{E-05}	0.9974	1.00	3.00

λ: Wavelength, [a]Based on six calibration curves LR: Linear regression , Sa: Standard deviation of intercept of regression line, Sb: Standard deviation of slope of regression line, R: Coefficient of correlation , x: atorvastatin concentration (μg ml^{-1}), A: Absorbance, ^{1}D: First order-absorbance, LOD: Limit of detection, LOQ: Limit of quantification

Table 2.Precision and accuracy of proposed methods

Method	λ (nm)	Added (μg ml^{-1})	Within-day			Between-day		
			Found±SD (μg ml^{-1})	Accuracy	Precision RSD%[a]	Found±SD (μg ml^{-1})	Accuracy	Precision RSD%[a]
UV	$A_{247\ nm}$	6	5.81 ± 0.159	-3.17	2.74	5.95 ± 0.241	-0.83	4.05
		13	13.3 ±0.356	2.31	2.68	13.4 ± 0.465	3.08	3.47
		19	19.2±0.341	1.05	1.78	19.3±0.261	1.58	1.35
1D	$^1D_{237\ nm}$	6	5.92 ± 0.192	1.33	3.24	6.04 ± 0.276	0.67	4.57
		13	13.1 ± 0.494	0.77	3.77	13.2 ± 0.516	1.54	3.91
		11	11.24±0.423	2.18	3.76	11.28±0.482	2.55	4.27
	$^1D_{261\ nm}$	6	6.06 ± 0.243	1.00	4.01	5.89 ± 0.187	1.83	3.17
		13	13.4 ± 0.445	3.08	3.32	13.3 ±0.326	2.31	2.45
		19	19.4±0.228	2.10	1.17	19.6±0.486	3.16	2.48

SD: Standard deviation of six replicate determinations, RSD%: Relative standard deviation, Accuracy:(%relative error)(found-added/addedx100)

Stability

Spectrophotometrically, to determine the stability of atorvastatin standard solutions in the refrigerator and at room temperature, atorvastatin solutions of 10, 15 and 20 μg ml^{-1} concentrations and stock solution were stored in the refrigerator and at room temperature for four days. Then, the stability measurements were carried out. The results were evaluated by comparing these measurements with those of standards and expressed as percentage deviation. The stability of atorvastatin solutions were determined by keeping them for three days in the refrigerator and for two days in room temperature. A significant change in concentration (recovery = 100 ± 3%) were not found under both conditions. In addition to this, stock solution was found to be stable for a week in refrigerator.

Specificity

The specificities of the two methods were investigated by observing interferences between atorvastatin and the excipients. Standard atorvastatin and drug formulation solutions were prepared and analyzed. No interference was found from tablet excipients at the selected assay conditions.

Assay sample preparation

The average tablet mass was calculated from the mass of tablets of Ator, Cholvast or Lipitor (30 mg atorvastatin tablet, which was composed of atorvastatin and some excipients). They were then finely ground, homogenized and portion of the powder was weighed accurately, transferred into a 100 mL brown measuring flask and diluted to scale with methanol. The mixture was sonicated for at least 10 min to aid dissolution and then filtered through a Whatman 42 paper. An appropriate volume of filtrate was diluted further with methanol so that the concentration of atorvastatin in the final solution was within the working range and then recorded against methanol.

Table 3. Results of analytical recovery studies by standard addition method (n=6)

Method	Amount taken (μg ml^{-1})	Amount added (μg ml^{-1})	Total amount found (μg ml^{-1})(Mean ±SD)	Recovery±RSD (%)
UV	5	2.5	7.55±0.10	103.0±1.32
		7.5	12.49±0.15	99.9±1.20
		12.5	17.25±0.28	98.0±1.62
$^1D_{237\ nm}$	5	2.5	7.49±0.11	99.6±1.46
		7.5	12.51±0.35	100.1±2.80
		12.5	17.48±0.13	99.8±0.743

Table 5.Statistical comparison (t-test) of the results obtained by proposed methods

Commercial preparation	Statistical Values	UV Method	^{1}D Method	t values
Ator	n	18	18	
	X	99.4	101.1	t_c=1.28
	SD	2.28	3.06	t_t=1.69
	CI	98.2-101.2	99.0-102.5	
Cholvast	n	18	18	
	X	98.7	101.1	t_c=1.42
	SD	2.50	3.10	t_t=1.69
	CI	97.3-101.2	100.0-102.5	
Lipitor	n	18	18	
	X	99.7	101.1	t_c=1.34
	SD	0.50	1.02	t_t=1.69
	CI	98.0-101.2	99.0-102.5	

n: Number of determination, X: Mean, SD: Standard deviation, CI: Confidence interval, t_c: Calculated F values, t_t: Tabulated t values, H_o: Hypothesis: no statistically significant difference exists between two methods, $t_t > t_c$: H_o hypothesis in accepted (α=0.05)

The percent analytical recovery values were calculated by comparing concentration obtained from the spiked samples with actual added concentrations. The mean recoveries of UV and ^{1}D spectrophotometry methods were 100.6 and 99.3%, respectively **(Table 4).**

Comparison of two spectrophotometric methods

The results show the high reliability and reproducibility of two methods. The best results obtained at 247 nm and 237 nm for zero- and first-order derivative spectrophotometric methods were statistically compared using the t-test. At 95 % confidence level, the calculated t-values do not exceed the theoretical values **(Table 5)**.

Therefore, there is no significant difference between zero- and first-order derivative spectrophometric methods. This is suggested that the two methods are equally applicable. The proposed methods are very effective for the assay of atorvastatin in tablets. The validity of the proposed methods was presented by recovery studies using the standard addition method. For this purpose, a known amount of reference drug was spiked to formulated tablets and the nominal value of drug was estimated by the proposed methods. Each level was repeated six times. The results were reproducible with low SD and RSD. No interference from the common excipients was observed.

CONCLUSION

In the present report, simple, rapid, sensitive, reliable, specific, accurate and precise UV and ^{1}D spectrophotometric method for the determination of atorvastatin in pharmaceutical preparations were developed and validated. The proposed methods can be used effectively, without separation and interference, for routine analysis of atorvastatin in pure form and its formulations and can also be used for dissolution or

similar studies. On the other hand, UV and ^{1}D spectrophotometric method are also suitable for analysis of sample during accelerated stability studies, routine analysis of formulations and raw materials.

Aknowledgment

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Conflict of Interest

The authors declare that they don't have any conflict of interest.

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