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Utilization of Ammonium Ceric Sulfate and Methyl Orange for Indirect Spectrophotometric Determination of Atorvastatin in Pharmaceutical Dosage Form

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Abstract

A simple, sensitive and reliable spectrophotometric method for determination of atorvastine (ASN) in pharmaceutical dosage form was developed by us. The method depends on oxidation-reduction reaction between atorvastatin and ammonium ceric sulfate (ACS) in acidic medium. The remaining unreacted ACS was allowed to react with methyl orange (MO) followed by measuring the remaining unbleached MO. Many parameters controlling this reaction have been studied and optimized. The method was validated over a concentration range of 3 µg/ml to 23 µg/ml by following ICH guideline. The limit of detection (LOD) and limit of quantitation (LOQ) were 0.83 µg/ml and 2.57 µg/ml respectively. The method was compared the reported method and it was found that there is no significance difference between the two methods the method was successfully applied to the quantitative determination of atorvastatin in its tablets.

***Keywords:** Atorvastatin; Ammonium ceric sulfate; Spectrophotometry; Methyl orange; Oxidation-reduction reaction; LOD; LOQ*

Introduction

Atrovastatin (ASN) is (3R,5R)-7-[2-(4-fluorophenyl)-3- phenyl-4-(phenylcarbamoyl)-5-(propan-2-yl)-1H-pyrrol-1-yl]-3,5-dihydroxyheptanoic acid (CAS no: 134523-03-8) (FIG. 1). It is antihyperlipidemic drug used in lowering cholesterol level a by inhibition of HMG-CoA (3-hydroxy-3-methyl- glutaryl-coenzymeA) reductase [1]. The role of this enzyme in synthesis of cholesterol is due to catalyzing the conversion of HMG-CoA to mevalonate [2]. Lowering cholesterol biosynthesis will lead to clear the LDP (low-density lipoprotein) in the blood by increased LDL receptors.

It prevents the synthesis of cholesterol through the inhibition of HMG-CoA reductase, this will prevent cholesterol production. Marked reduction in total cholesterol, low-density lipoprotein cholesterol and plasma triglycerides have been seen for long term oral administration of ASN. Many methods have been published for analysis of ASN by different analytical techniques, spectrophotometry [3-6], spectrofluorometry [7], high performance liquid chromatography [8,9], LC-

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MS [10-12]. Most of the published methods used sophisticated instruments and or procedures. Because of the importance of ASN in treatment of heart disease and it present in many pharmaceutical dosage form, there is a need to develop a simple, sensitive and robust method. This method used simple instrument and procedure to be used in routine determination of ASN.

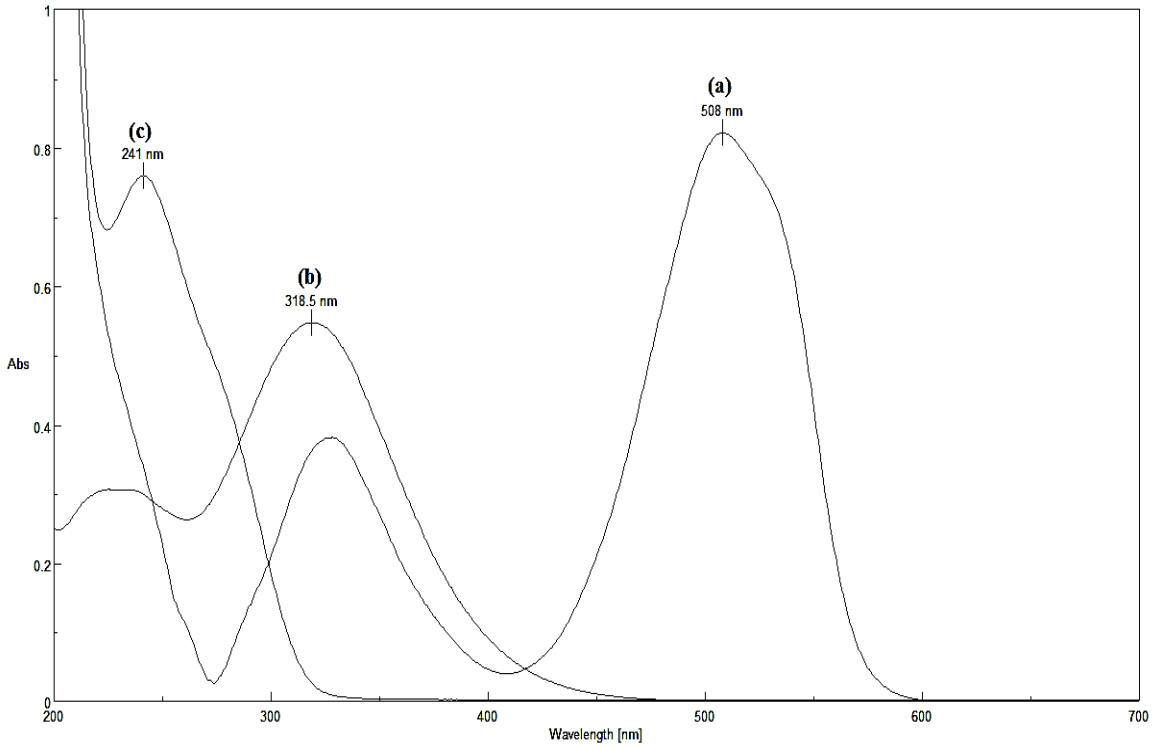

FIG. 1. **(a). Absorption spectrum of remaining MO after reaction with unreacted ammonium ceric sulfate (20 µg/ml atorvastatin), (b) absorption spectrum of ammonium ceric sulfate in acidic medium and (c) absorption spectrum of atorvastatin (20 µg/ml) in acidic medium.**

Experimental

Apparatus

Jasco V-630 UV-VIS Spectrophotometer with Spectra Manager™ II software.

Chemicals and reagents

Deionized water (PureLab Flex) was used throughout the procedures. Methyl orange (MO) was purchased from SD Fine-Chem Limited (SDFCL), Mumbai, Maharashtra, India. Ammonium ceric sulfate dihydrate (ACS) was purchased from Sigma-Aldrich, Steinheim, Germany. Sulfuric acid 98% was purchased from El-NASR Company, Egypt.

Pharmaceutical formulations

ATOR 10 tablets labeled to contain 10 mg of atorvastatin, produced by EIPICO were purchased from the local market.

Preparation of working solutions

Working standard solution of atorvastatin was prepared directly by dissolving atorvastatin in methanol to yield a concentration of 1 mg/ml and stored at -20°C. Working solution of MO was prepared directly by dissolving MO in water to yield a 0.001 M aqueous solution and stored at 4°C. 0.5 M and 5 M sulfuric acid were prepared by dissolving the appropriate

volumes of 98% sulfuric acid in water. Working solution of ammonium ceric sulfate was prepared directly by dissolving it in 0.5 M sulfuric acid to yield a 0.001 M aqueous solution which was also stored at 4°C.

Preparation of calibration standards

Aliquots of 0.030 ml to 0.230 ml of working solution of atorvastatin were transferred to a series of 10 ml volumetric flasks followed by 0.5 ml of 5 M sulfuric acid. 1.3 ml of ACS working solution is added followed by occasional shaking for 5 min. Then 0.3 ml of working solution of MO was added followed by 5 min of occasional shaking then the volume was completed with water to the mark to yield calibration standards containing atorvastatin in the range of 3 µg/ml to 23 µg/ml.

Preparation of pharmaceutical preparation

Ten tablets were crushed, powdered and homogenized then extracted with 50 ml methanol, filtered through a dry funnel and filter paper. The filtrate was then filtered through 0.2 um Millipore filter, transferred to 100 ml volumetric flask and the volume was completed with methanol and stored at 4°C. A 0.07 ml aliquot was taken to yield a 7 µg/ml solution of the drug.

General procedure

Aliquots 0.2 ml of atorvastatin working solution was transferred to a 10 ml volumetric flask followed by 4 ml of deionized water and 1 ml of 5 M sulfuric acid. 2 ml of ammonium ceric sulfate working solution is added followed by occasional shaking for 15 min at room temperature. Then 0.5 ml of working solution of MO was added followed by 10 min of occasional shaking at room temperature then the volume was completed with water to the mark. This solution is scanned in the UV-Visible spectrophotometer against a reagent blank.

Results and Discussion

Explanation of reaction mechanism

MO was found to absorb maximally at wavelength 508 nm. ACS is strong oxidizing agent in acidic medium. It able to be oxidized MO with bleaching the color. The reaction between ASN and ACS belongs to oxidation-reduction reactions. Addition of acidified ACS in known excess oxidized ASN and the reaming un reacted ACS will bleach the color of MO. High concentration of ASN will consume more ACS and increase of the amount of MO remained. As conclusion it was found that there is a linear relationship between the concentration of ASN and absorbance intensity of MO at wavelength 508 nm. FIG. 1 shows spectral characterizations of ASN, characterizations and MO at wavelength 508 nm.

First of all, the oxidization of atorvastatin by ACS was studied and it was found that the solution containing the drug possessed an absorbance for MO at λ 508 nm when measured against a blank containing only ASC and MO (FIG. 1).

Different factors affecting this indirect measurement were studied at room temperature in order to determine the optimal conditions for assay procedure. These include the optimal volume of methyl orange, the optimal volume of ACS, volume of acid, time to oxidize atorvastatin, time to bleach MO and stability of color.

It was found that maximum absorbance (A) for MO at λ 508 nm when we use 0.3 ml of MO, 1.3 ml of ACS in presence of 0.5 ml 5 M sulphuric acid. The required time for reaction is not more than 5 min and color stability obtained up to 1 h. Under the optimized experimental conditions, the method has been validated for linearity, limits of detection and quantitation, accuracy and precision according to ICH guidelines [13]. From the resulting peaks of calibration, the absorbance values were plotted versus the corresponding concentrations (µg/ml) and the calibration graph was obtained **(**FIG. 1). Calibration graph

was linear over the range of 3 µg/ml to 23 µg/ml. The coefficient of determination (r^2) was 0.999. The calibration graph had a reliable reproducibility across the calibration range. The following regression equation was computed:

$$Y=0.04991 \times X-0.09768 \quad r=0.999$$

Where X is the concentration in µg/ml, Y is the absorbance and r is the regression coefficient (FIG. 1).

Based on residual standard deviation of the regression line, the LOD and LOQ were calculated and found to be 0.83 µg/ml and 2.57 µg/ml respectively (TABLE 1).

TABLE. 1. **Linearity data of atorvastatin by the proposed indirect spectrophotometric method.**

Linearity range	3 µg/ml to 23 µg/ml
Slope	0.048
Intercept	-0.079
Correlation coefficient	0.999
r^2	0.999
SE of slope	6.545×10^{-4}
Confidence limit of slope	$0.048 \pm 1.60 \times 10^{-3}$
SE of intercept	9.351×10^{-3}
Confidence limit of intercept	-0.079 ± 0.023
Residual SD ($S_{y/x}$)	0.012

The method was found to have acceptable accuracy (TABLE 2). Statistical comparison of the performance of the proposed method with that of the official method showed that there was no significant difference in their accuracy and precision as shown by the results of student's t-test and variance ratio F-test respectively (TABLE 3). Intra-day and inter-day precision testing indicate that the repeatability of the proposed method is good as indicated by small value of %RSD (TABLE 4).

The proposed indirect spectrophotometric method was applied to the assay of atorvastatin in ATOR 10 mg tablets to demonstrate its efficiency in the analysis atorvastatin in pharmaceutical dosage form for quality control testing. The concentration of atorvastatin was calculated using the regression equation that was obtained from the proposed method. To validate the application on pharmaceutical dosage form, standard addition technique was applied. The mean percentage recoveries of taken atorvastatin was 97.11 ± 0.45 and the mean percentage recoveries of added atorvastatin was 99.28 ± 0.91 (TABLE 5).

TABLE. 2**. Accuracy results for atorvastatin by the proposed indirect spectrophotometric method.**

Concentration taken (µg/ml)	Recovery percentage*	Mean ± SD	%RSD	RSE	Variance
4	99.37%	99.28 ± 0.50	0.50%	0.22%	0.25
7	99.61%				
12	98.81%				
16	98.74%				
21	99.89%				

*Average of three different determinations.

TABLE. 3. **Statistical comparison of the performance of the proposed method with that of the official method for the analysis of atorvastatin in pure form.**

Item	Proposed	Official [14]
Mean accuracy ± SD	99.28 ± 0.50	99.74 ± 0.73
%RSD	0.50%	0.73%
%RSE	0.22%	0.33%
N	5	5
Variance	0.25	0.53
t-test (2.31)	1.15	
F-test (6.39)	2.11	

Values in parenthesis represent the tabulated values of t and F at p=0.05.

TABLE. 4. **Results of testing the precision of the proposed indirect spectrophotometric method for the analysis of atorvastatin in pure form.**

Intraday precision (%RSD)	0.54%
Inter-day precision (%RSD)	0.80%

TABLE. 5. **Assay of atorvastatin it its tablets by the proposed indirect spectrophotometric method using standard addition technique.**

Item	Taken concentration (µg/ml)	Added concentration (µg/ml)	Recovery percentage*
	7	5	98.36%
		7	98.71%
		9	100.54%
		11	99.22%
		13	100.24%
		15	98.62%
Mean ± SD	97.11 ± 0.45		99.28 ± 0.91
% RSD	0.46%		0.92%
% RSE	0.27%		0.37%
Variance	0.20		0.83

* Average of three different determinations.

Conclusion

The previous results showed the benefit of using the ACS/MO oxidation-reduction system spectrophotometric determination of ASN. The simplicity, sensitivity and economically of the method are the main advantages of this method. The developed method can be used in routine analysis of ASN in bulk powder as well as applied in quality control laboratories for the routine analysis of the investigated drugs in raw materials, in pharmaceutical formulations.

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