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Spectrophotometric Methods for Estimation of Atorvastatin Calcium Form Tablet Dosage Forms

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Abstract: Quantitative estimation of poorly water-soluble drugs involves use of organic solvents. Major drawbacks of organic solvents include high cost, volatility and toxicity. In the present investigation, hydrotropic solubilization is employed to enhance the aqueous solubilities of poorly water-soluble drugs like Atorvastatin Calcium in tablet dosage forms. This method utilizes 2.0 M urea solution as, hydrotropic solubilizing agent. In the urea solution Atorvastatin Calcium shows maximum absorbance at 240 nm. The 2.0 M urea solution does not show any interference with the sampling wavelength. Another method is formation of green color complex between the drug Atorvastatin Calcium and 0.3 % w/v ferric chloride and 0.02 % w/v potassium ferricyanide. The green colored complex shows the maximum absorbance at 787 nm. The hydrotropic agent and additives used in the manufacture of tablets did not interfere in the analysis. The results of tablet analysis were found be in range of 99.26 to 100.12% with standard error values of 0.2728 and 0.2082 by Hydrotropy and Colorimetry respectively. The results of analysis of both methods were validated statistically following ICH Q2A (R1) guidelines. Both methods were found to be useful for accurate, sensitive, selective, precise and robust analysis of Atorvastatin from marketed formulations. After optimization, methods will be useful for analysis of Atorvastatin in biological fluids.

Key Words: Atorvastatin Calcium, Urea, Hydrotropy, Colorimetry.

Introduction

Atorvastatin Calcium is chemically described as [R-(R*, R*)]-2-(4-fluorophenyl)-β, ∂ dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenyl amino) carbonyl]-1H-pyrrole-1-heptanoic acid calcium salt trihydrate is an anti hyperlipoproteinemic agent act by inhibiting HMG - CoA reductase (1-2). Many analytical methods like UV spectroscopy, HPLC were reported for determination of Atorvastatin Calcium alone and combination with other antihypertensive drugs (3-8). In the analysis of Atorvastatin, major problem is solubilization of Atorvastatin in most of solvents during analysis (9). Quantitative estimation of poorly water-soluble drugs involves use of organic solvents. Major drawbacks of organic solvents include high cost, volatility and toxicity. In the present investigation, hydrotropic solubilization is employed to enhance the aqueous solubilities of poorly water-soluble drugs like Atorvastatin Calcium in tablet dosage forms. In a proposed method this problem has been solved by using 2 M urea solution. Highly sensitive and economic colorimetric method is not reported for determination of Atorvastatin. Hence in this communication we have reported new UV spectrophotometric methods for determination

Atorvastatin Calcium using hydrotropy and colorimetry.

Experimental

Materials:

Instrument:

Spectrophotometric analysis was carried out on a JASCO UV-spectrophotometer 530 using a 1 cm quartz cell. The instrument settings was zero order derivative mode and band width of 2.0 nm in the range of 200–350 nm and 400-800 nm.

Reagents and Chemicals:

Atorvastatin Calcium supplied by Cipla Ltd. India. All chemicals were analytical grade obtained from SD fine chemicals. Water purified by glass distillation apparatus.

Preparations of Standard Drug Solutions and Reagents:

For hydrotropic solubilization 20 mg of pure Atorvastatin Calcium was dissolved in 50 ml of 2.0 M urea solution and stirred for 15 minutes and the final volume of both solutions was made up to 100 ml with distilled water (10). The solution was filtered through Whatmann filter paper No. 41. This solution was further diluted with distilled water to prepare working concentrations of 100 µg/ml of Atorvastatin Calcium. For the colorimetric method standard solution of Atorvastatin Calcium was prepared in solvent system methanol: water (60:40) about 10 mg of drug was dissolved in 70 ml of solvent system sonicated for 10 minutes and final volume was made up to 100 ml to get working solution of 100 µg/ml. Ferric Chloride was prepared by dissolving 300 mg in 100 ml of 0.5 M hydrochloric acid and potassium ferricyanide was prepared by dissolving 20 mg in 100 ml of distilled water.

Methods:

Preliminary Solubility Studies of Drugs:

Solubility of Atorvastatin Calcium was determined at 28±1°. An excess amount of drug was added to 2M urea solution in vials. The vials were shaken mechanically for 12 h at 28±1°, in a mechanical shaker. These solutions were allowed to equilibrate for the next 24 hours, and then centrifuged for 5 minutes at 2000 rpm. The supernatant of each vial was filtered through Whatmann filter paper No. 41. The filtrates were diluted suitably, and analyzed spectrophotometrically against corresponding solvent blank.

Method 1: Spectroscopic method employing hydrotropic solubilization.

From the overlain spectra of the drug Atorvastatin Calcium and 2.0 M urea (Figure 1), it was found that the urea used does not interfere with the sampling wavelength. Therefore 2.0 M urea is used for the solubilization of drug (11). Different aliquots of the standard solutions were taken in series of volumetric flasks to prepare the concentrations ranging from 5-45 µg/ml and volume was made up to mark with water. These concentrations were scanned in the range 200-400 nm range absorbances of each concentration taken at 240 nm. Calibration curve was prepared by plotting absorbance against concentration.

Procedure for analysis of tablet formulation:

Twenty tablets were weighed and ground to a fine powder. Tablet powder equivalent to 30 mg Atorvastatin Calcium was weighed and transferred to a 100 ml volumetric flask 70 ml of 2.0M urea solution was added to the flask and stirred for 15 min to dissolve the drug and the final volume was made up to 100 ml with distilled water. The solution was filtered through Whatmann fitter paper No. 41 and the first few ml were rejected. The filtrate was diluted suitably with distilled water to get 10 µg/ml of Atorvastatin Calcium. The absorbance at 240 nm was measured and the amount of drug present in the sample solution was obtained from the slope and intercept values obtained from the calibration curve (Table 1). From these, concentrations, the composition of the tablet was obtained. The results of analysis of tablet formulations are recorded in Table 2. After 48 hours, the solutions were reanalyzed to determine chemical stability and precipitation, if any.

Method 2: Spectroscopic method employing colorimetric method.

Overlain spectra of reagent and the drug reagent colour complex is shown in Figure 2, which shows that change in the colour is observed by addition of drug to reagent. The figure shows maximum absorbance at 787 nm which is selected as sampling wavelength. Different aliquots of the standard drug solutions were taken in series of 10 ml volumetric flasks to prepare the concentrations ranging from 4-24 µg/ml to each flask 2 ml of 0.3 % w/v of ferric chloride and 2 ml of 0.02 % w/v potassium ferricyanide was added and volume was made up to mark with water after 30 minutes the green coloured stable complex was formed. These solutions were analyzed in 400-800 nm range and spectras were recorded. The absorbance of each concentration at 787 nm is plotted against concentration which gives calibration curve.

Procedure for analysis of tablet formulation:

Twenty tablets were weighed and ground to a fine powder. Tablet powder equivalent to 10 mg Atorvastatin Calcium was weighed and transferred to a 100 ml volumetric flask to this 70 ml of solvent system

methanol: Water (60:40) was added. This solution was sonicated for 10 min and volume was made up to mark then solution was filtered through the Whatmann fitter paper No. 41 first few ml is rejected. This solution was further diluted to obtain the concentration of 10 µg/ml of Atorvastatin Calcium. To this solution 2 ml of 0.3 % w/v of ferric chloride and 2 ml of 0.02% w/v of potassium ferricyanide was added solution was kept aside, after 30 minutes, stable green colored complex was formed. Then solution was analyzed in 400-800 nm range and from absorbance at 787 nm was recorded. Concentration of solution was calculated from the slope and intercept values obtained from calibration curve (Table 1). The results of analysis of tablet formulation are reported in Table 2.

Method Validation:

The method was validated according to ICH Q2B R1 guidelines for validation of analytical procedures in order to determine the linearity, sensitivity, precision, robustness and accuracy for the analyte (12). Accuracy and specificity of analysis was determined by performing recovery studies by spiking different concentrations of pure drug in the preanalyzed tablet sample. Results of validation parameters are reported in Table 3.

Table 1: Optical Characteristics of Developed Methods

Parameters	Atorvastatin Calcium	
	Hydrotropy	Colorimetry
Beer's law limit (µg/ml)	5-45	4-24
Regression equation	Abs = A + B * Conc.	Abs = A + B * Conc.
Slope (B)	0.0317	0.0325
Intercept(A)	0.0998	0.0862
Correlation coefficient	0.9999	0.9992

Conc.: Concentration of analyte

Table 2: Results of Analysis of Marketed Tablet Formulation with Statistical Evaluation

Method	Label claim mg/tablet	%Label claim estimated (Mean ± S.D.) (n=6)	%CV	SE
Hydrotropy	10	99.63 ±0.4726	0.4743	0.2728
Colorimetry	10	99.97±0.2082	0.1202	0.2082

S.D.: Standard Deviation; CV: Coefficient of Variance; SE: Standard Error.

Table 3: Results of Recovery Studies

Method	Amount of drug taken (mg)	Drug added (spiked) (mg)	%Recovery estimated (Mean ± S.D.) (n=3)	% CV	SE
Hydrotropy	10	8	99.9±0.69	0.69	0.39
		10	99.9±0.76	0.76	0.43
		12	99.9±0.91	0.92	0.53
Colorimetry	10	8	99.7±1.38	1.38	0.80
		10	99.3±0.77	0.77	0.44
		12	99.1±0.68	0.70	0.39

S.D.: Standard Deviation; CV: Coefficient of Variance; SE: Standard Error.

Table 4: Limit of Detection and Limit of Quantitation (n-9)

Method	Limit of Detection	Limit of Quantitation
Hydrotropy	0.1025	0.3789
Colorimetry	0.9452	0.2546

Table 5: Result of Repeatability (n=9)

Method	Amount found (mean% ± S.D.)	Accuracy, Bias (%)	SE	% CV
	Intraday Precision			
Hydrotropy	99.48± 0.29	-0.6	0.1674	0.29
Colorimetry	99.51± 0.24	-0.5	0.1385	0.25
	Inter Day Precision			
Hydrotropy	99.55± 1.20	-0.5	0.6939	1.20
Colorimetry	99.51± 0.24	-0.6	0.6939	0.36

S.D.: Standard Deviation; CV: Coefficient of Variance; SE: Standard Error.

Table 6: Result of Intermediate Precision (n=3)

Method	Amount found (mean% ± S.D.)	SE	% CV
	Analyst 1		
Hydrotropy	99.69± 0.72	0.4157	0.72
Colorimetry	99.46± 0.45	0.2598	0.45
	Analyst 2		
Hydrotropy	99.22± 0.66	0.3810	0.66
Colorimetry	99.95± 0.28	0.6634	0.28

S.D.: Standard Deviation; CV: Coefficient of Variance; SE: Standard Error.

Table 7: Results of Robustness Studies of Hydrotropy Method (n=9)

Robustness Parameter	Label claim (mg/tab)	% Label claim estimated (Mean ± R.S.D.)	R.S.D.
Analysis using 2.5 M urea solution	10	100.50 ± 1.4179	1.4109

R.S.D.: Relative Standard Deviation.

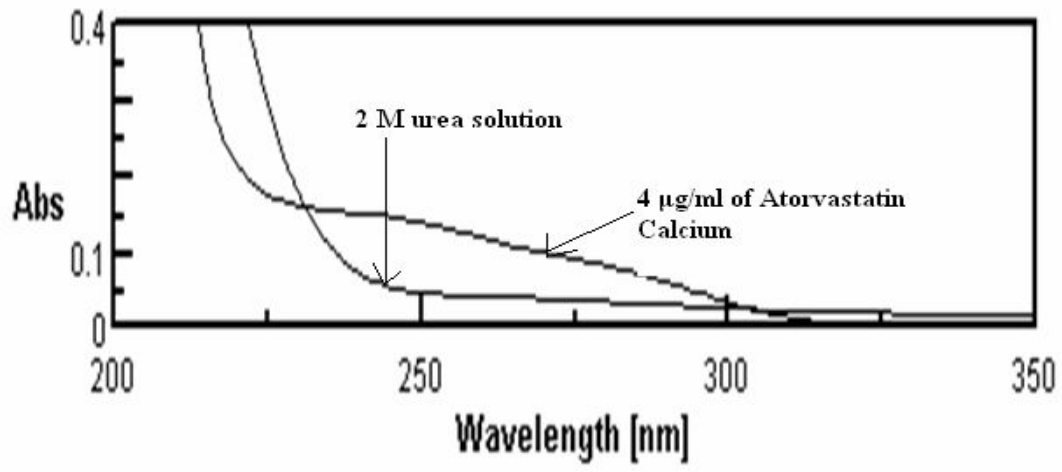

Figure 1: Overlain Spectra of 5 µg/ml of Atorvastatin Calcium in Urea and 2.0 M Urea Solution.

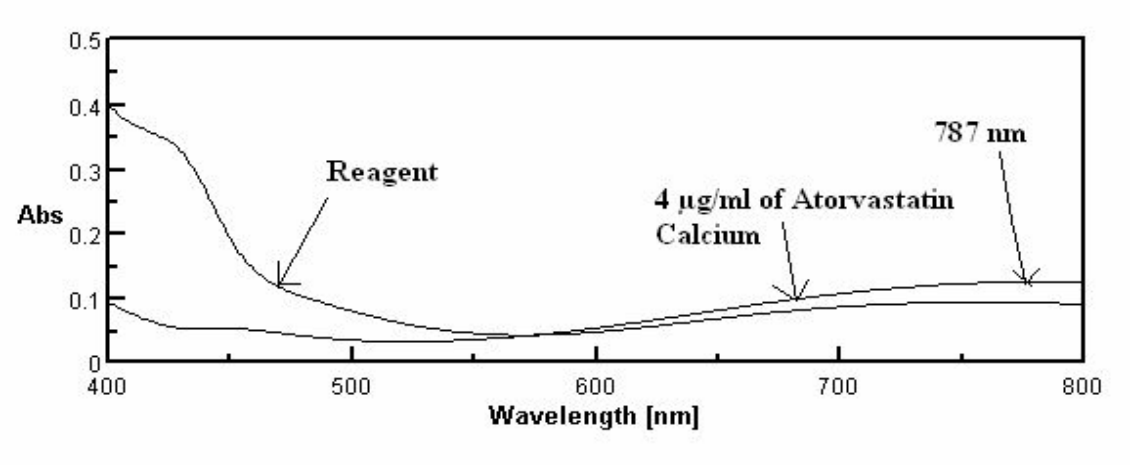

Figure 2: Overlain Spectra of 4 µg/ ml of Atorvastatin Calcium and Reagent.

Results

Quantitative estimation of poorly water-soluble drugs involves use of organic solvents. Major drawbacks of organic solvents include high cost, volatility and toxicity. In the present investigation, hydrotropic solubilization is employed to enhance the aqueous solubilities of poorly water-soluble drugs like Atorvastatin Calcium in tablet dosage forms. The results of solubility studies indicated that enhancement in aqueous solubility of Atorvastatin Calcium in 2.0 M urea solution was more than 6-7 folds as compared to their solubilities in distilled water. Therefore, this solution was employed to extract Atorvastatin Calcium from the fine powder of tablet formulation and thus analysis will become easier one.

The colorimetric method was optimized for colorimetric reagents selection, their concentration, sequence of additions, time for color development etc (13-14). Addition of ferric chloride and potassium ferricyanide to sample of Atorvastatin leads to development of highly intense green colored complex.

The result of analysis of tablet formulation by both methods showed % relative standard deviation values in the range of 0.5859 to 0.9992 for ATR, which indicates repeatability of the method. The results indicated excellent recoveries ranging from 98.52 to 101.1 % for ATR. Recoveries obtained for the drug do not differ significantly from 100 %, which showed that there was no interference from common excipients used in the formulation. The results of limits of detection and quantitation were within limits.

Discussion

It is evident from results of validation studies for both methods that methods are accurate, sensitive, selective, precise and robust for spectroscopic estimation of Atorvastatin. Moreover the method is economic, simple and rapid, hence can be employed for routine analysis in quality control laboratories for estimation of Atorvastatin form marketed formulations. After optimizing these methods to estimate Atorvastatin from biological fluids, these methods can be used in Clinical and Bioequivalence studies.

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List of Non-standard Abbreviations Used

- **rpm**: revolutions per minute
- **ATR**: Atorvastatin Calcium
- **Abs**: Absorbance
- **Conc.**: Concentration
- **S.D.**: Standard Deviation
- **CV**: Coefficient of Variance
- **SE**: Standard Error.
- **R.S.D.**: Relative Standard Deviation.
- **n**:Number of times analysis is repeated

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