



Development and Validation of First Order Derivative Spectrophotometric Method for Simultaneous Estimation of Atorvastatin Calcium and Aspirin in Capsules

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ABSTRACT

A simple UV spectrophotometric method using first order derivative technique was developed for the simultaneous estimation of atorvastatin calcium and aspirin in capsules. The atorvastatin calcium and aspirin stock solutions were prepared in (50: 50 v/v) methanol: water and scanned in UV- region for first order derivative spectrum. The zero crossing point for atorvastatin calcium and aspirin was 243 nm and 276 nm respectively. Linearity was established over the concentration range of 6-14 µg/ml and 20-100 µg/ml for atorvastatin calcium and aspirin with correlation coefficient (r^2) value 0.998 and 0.999 respectively. The method was validated according to ICH guidelines for validation parameters like accuracy, precision, limit of detection and limit of quantification. They found to be within limits. The method was successfully applied for quantitative estimation of atorvastatin calcium and aspirin in capsules.

Keywords: Atorvastatin calcium, Aspirin, Derivative spectroscopy, Zero crossing point, Correlation coefficient, ICH guidelines.

INTRODUCTION

Atorvastatin Calcium (Figure 1) is: [(βR, ds)-2-(4-fluorophenyl)-β, δ-di hydroxy-5-(1-methylethyl)-3-phenyl-4 [(phenyl amino) carbonyl]-1-heptenoic acid calcium salt (ATV). It is statin used for lowering blood cholesterol. It is a selective competitive inhibitor of the enzyme HMG-CoA reductase, which catalyses the conversion of HMG-CoA to mevalonate, an important rate limiting step in cholesterol biosynthesis. Aspirin (ASP) is chemically known as acetyl salicylic acid and is used as non-steroidal anti-inflammatory and analgesic drug [1,2].

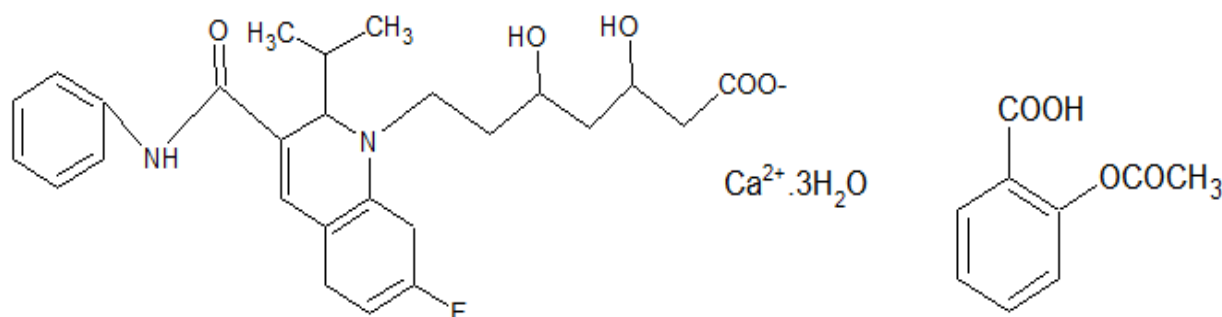


Figure 1: Structure of atorvastatin calcium and aspirin

Both atorvastatin calcium and aspirin are freely soluble in methanol, slightly soluble in ethanol and very slightly soluble in distilled water. As per literature HPLC and UV spectrophotometric methods are available for simultaneous estimation of atorvastatin calcium and aspirin in marketed formulations [3-11]. To the best of our knowledge there is no derivative spectrophotometric method reported for determination of ATV and ASP simultaneously. Therefore, an attempt has been made to develop a simple, derivative spectrophotometric method for analysis of these two drugs. Derivative spectroscopy uses first or higher derivatives of absorbance with respect to wavelength for qualitative analysis and for quantification. The spectra obtained in derivative mode give high resolution and differentiation which makes easy for multicomponent analysis of mixtures of components [12,13].

Chemicals and reagents

Atorvastatin calcium and Aspirin are the gift samples from Aurobindo pharmaceuticals, Hyderabad. Methanol (Merck specialities private limited), water (SDFCL), sodium hydroxide (SDFCL) used are of analytical grade.

Instruments

UV-Visible Spectrophotometer (Shimadzu UV-1800 (UV probe 4.0 software), Digital balance (Shimadzu BL-220H), Ultra sonic bath sonicator (PCI Analytics 6.5 li200H), Refrigerated centrifuge (Eltek RC4100F) were used in analytical work.

Marketed formulation

AZTOR ASP capsules containing 10 mg of atorvastatin calcium and 75 mg of aspirin manufactured by Sun pharmaceuticals Ltd. were purchased from local market.

Selection of solvent

Solvent selection is the first step involved in the method development. Solvent is selected based on the solubility of the drugs. Absorption characteristics of 10 µg/ml standard solution of both drugs were checked in water, methanol, phosphate buffer pH 7.4, 0.1 N NaOH and methanol: water (50: 50). The overlay absorption spectrum of two drugs in all the solvents is shown in Figure 2.

Standard solutions

Standard stock solutions of atorvastatin calcium and aspirin (1000 µg/ml) were prepared separately in methanol: water (50: 50 v/v). Working standard solutions of these two drugs (100 µg/ml) were obtained by dilution of the respective stock solutions in methanol: water (50: 50 v/v).

Spectral characteristics and wavelength selection

The absorption spectra of 10 µg/ml of atorvastatin calcium and 20 µg/ml in the diluent were recorded by scanning in the UV wave length range 200-400 nm and overlay zero order (D^0) spectrum is shown in Figure 1. Zero order spectrums (D^0) were converted to first derivative spectra (D^1) using delta lambda 1.0 and scaling factor 4.0. The zero crossing point of atorvastatin and aspirin was found to be 246 nm and 276 nm respectively. Overlay first order derivative spectrum was shown in Figure 3.

Selection of analytical concentration ranges

Calibration standards at five levels were prepared by diluting the standard stock solution in the concentration range of 6-14 µg/ml for atorvastatin calcium and 20-100 µg/ml for aspirin respectively. The absorbance of these solutions was measured at their zero crossing point wavelengths.

Method validation

The method developed was validated for linearity, precision, accuracy, specificity, ruggedness, robustness, limit of detection, limit of quantification and robustness according to the ICH Guidelines [14].

Linearity and range

The linearity of analytical method is ability to obtain test results that are directly proportional to the concentration of analyte in the sample. The range of analytical method is the interval between upper and lower concentration (amounts) of analyte that have been demonstrated with suitable level of precision, linearity and accuracy.

Series of calibration standards were prepared in the concentration range 6-14 µg/ml of ATO and 20-100 µg/ml of ASP. The absorbance of these solutions was measured at 246 nm and 276 nm against solvent blank respectively and D^1 (first order derivative) absorbance values were recorded. A graph of concentration versus absorbance was plotted and correlation coefficient (r^2) was reported.

Limit of detection (LOD) and limit of quantification (LOQ)

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantified as an exact value. Quantification limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.

The sensitivity of proposed method for measurement of atorvastatin and aspirin was estimated in terms of LOD & LOQ determined using the standard deviation of the response and slope method.

Precision

The precision of an analytical procedure defines the degree of closeness of agreement between a series of measurements obtained from multiple samplings of homogenous sample under prescribed conditions. Precision of the method was reported as RSD% at different levels- repeatability, Intra-day precision and Inter-day precision.

Repeatability was evaluated by the analysis six replicates of 10 µg/ml of atorvastatin calcium and 30 µg/ml of aspirin for checking the variation of results on the same day. The intra-day and inter-day precision of the proposed method was determined by analyzing samples 32 µg/ml, 40 µg/ml and 48 µg/ml for aspirin and 6 µg/ml, 8 µg/ml, 10 µg/ml for atorvastatin, three replicates of each sample as a batch in a single assay on the same day and three consecutive days for inter-day precision.

Accuracy

Recovery studies were carried out by using standard addition method at three levels, 80%, 100% and 120% for ATV calcium (8 µg/ml, 10 µg/ml and 12 µg/ml) and ASP (60 µg/ml, 75 µg/ml and 90 µg/ml). At each level, the determination was done in triplicate and the amount of drug recovered was calculated and reported.

Analysis of fixed dose combination capsules

Twenty capsules of fixed dose combination capsules were weighed and emptied. The powder quantity equivalent to one capsule was transferred

to 100 ml volumetric flask containing 25 ml of solvent and sonicated for 5 min. The solution was centrifuged at 2500 rpm for 15 min and filtered using whatman filter paper. Sample stock solution was further diluted to get a solution containing 10 µg/ml of atorvastatin calcium and 75 µg/ml of aspirin. The absorbance of resulting solution was measured at 246 nm and 276 nm against solvent blank and %purity was calculated.

RESULTS AND DISCUSSION

Development of simple and accurate UV-spectrophotometric methods offers cost effective and time saving alternative to HPLC method of analysis for routine quantitative determination of samples. UV Spectroscopic method in derivative mode was developed and optimized for analysis of atorvastatin and aspirin simultaneously in capsules.

Derivatization of spectral data obtained in zero order spectra was done in order to improve resolution of the broad band peaks of atorvastatin and aspirin.

Solvent selection

Atorvastatin calcium and aspirin solubility were tested in water, methanol, phosphate buffer pH 7.4 and methanol: water (50: 50). Intensity of absorption of both drugs was good in methanol: water (50: 50 %v/v). This mixture of two solvents was selected for further analysis.

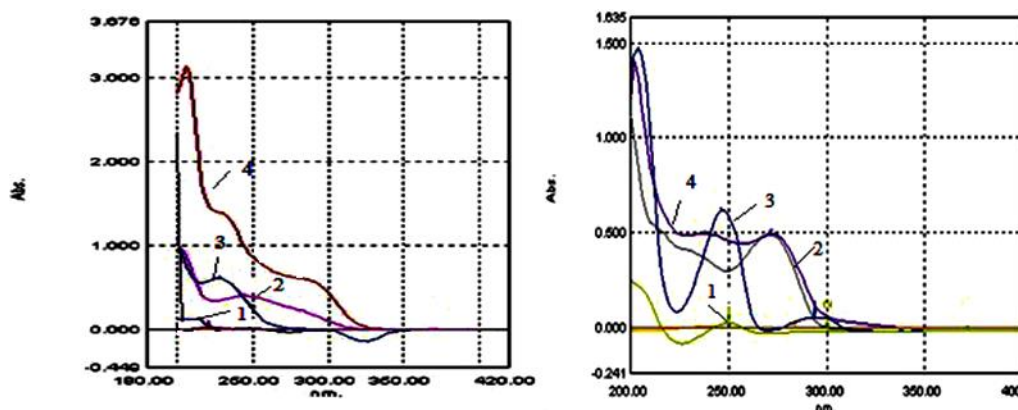


Figure 2: Overlay absorption spectrum of atorvastatin calcium and aspirin in water (1), methanol and water (2), methanol (3) and phosphate buffer (4)

The zero crossing point of atorvastatin and aspirin in their first derivative spectra was selected as wave length for development of method. They were found to be 246 nm and 276 nm and the respective derivative spectra are shown in Figure 3.

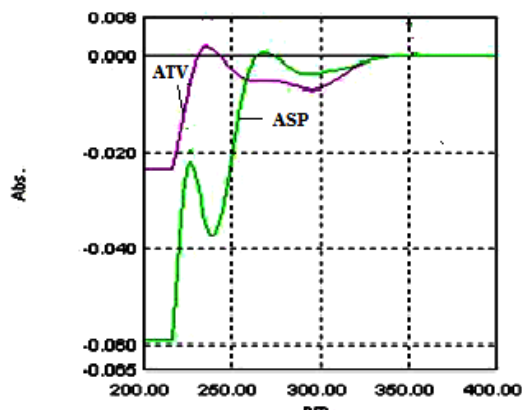


Figure 3: Overlay first order derivative (D^1) spectrum of atorvastatin calcium and aspirin in methanol: water

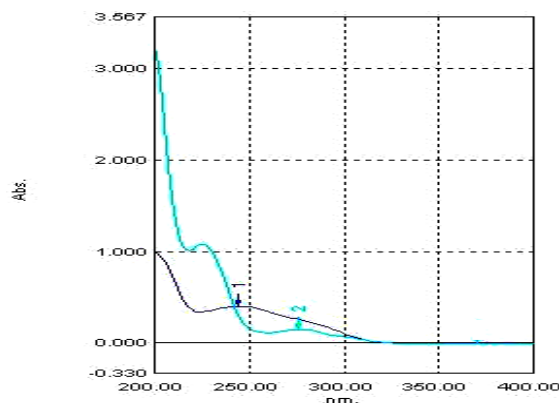


Figure 4: Overlay absorption spectrum of Atorvastatin calcium (1) and Aspirin (2)

Linearity

Linearity of the method was established from calibration curves constructed with the standard solutions of atorvastatin and aspirin in the concentration range 6-14 µg/ml and 20-100 µg/ml (Figures 4-8 and Table 1). The absorbance of the drug was plotted against the corresponding concentration. Linearity was proved by using linear regression analysis and calibration curves generated were found to be linear over the selected range, with coefficient of determination values (r^2) 0.999. The concentration of drugs in the formulation samples were calculated from the resulting regression equations.

Table 1: Linearity data for atorvastatin calcium and aspirin

S. No.	Concentration (µg/ml)		Absorbance*	
	ATV	ASP	ATV at 246 nm	ASP at 276 nm
1	6	20	0.225	0.137
2	8	40	0.298	0.246
3	10	60	0.387	0.401
4	12	80	0.463	0.558
5	14	100	0.562	0.716

*Average of three determinations

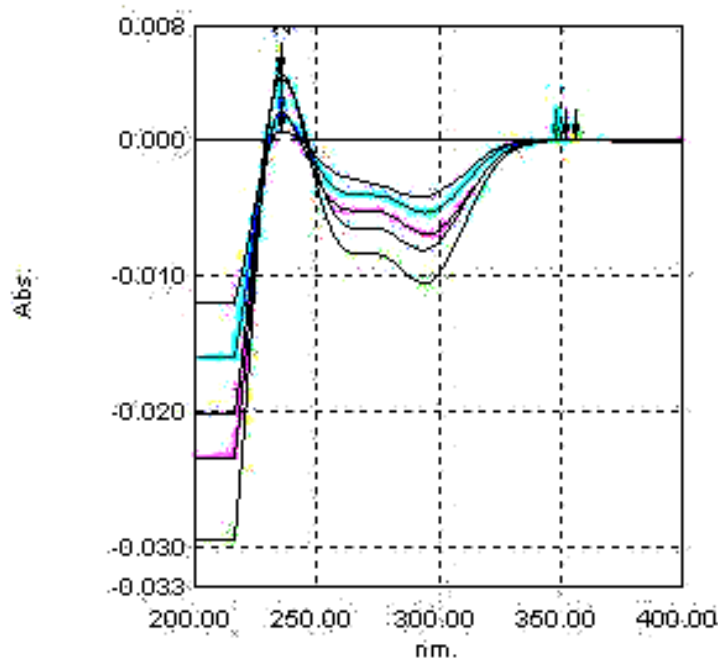


Figure 5: Overlay D¹ spectrum of atorvastatin calcium (6-14 µg/ml)

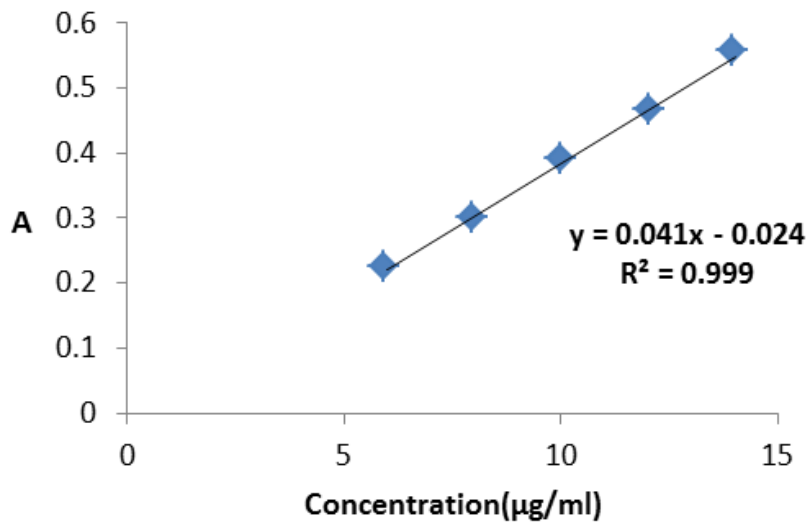


Figure 6: Standard calibration curve of atorvastatin calcium

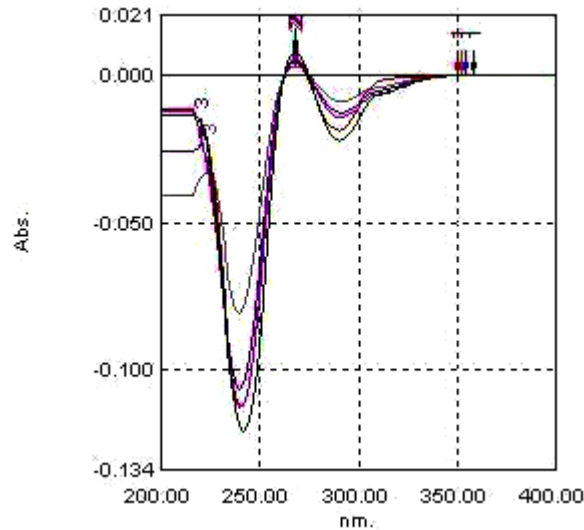


Figure 7: Overlay D¹ spectrum of aspirin (20-100 µg/ml)

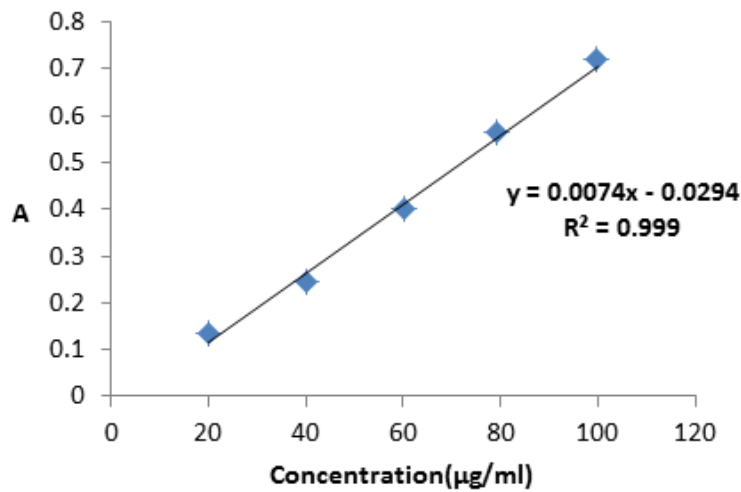


Figure 8: Standard calibration curve of aspirin

LOD and LOQ

The calculated LOD and LOQ values of atorvastatin were 0.41 µg/ml and 1.219 µg/ml and for aspirin 0.345 µg/ml and 1.9 µg/ml respectively, indicate the sensitivity of the method.

Accuracy and Precision

The results of intra- and inter-day precision determined by analyzing three replicates of standard samples at three levels on different days are summarized in Tables 2 and 3. The recovery studies data has proven the accuracy of the developed method for analysis marketed formulation. The results are shown in Table 4.

Table 2: Repeatability data of atorvastatin calcium and aspirin

S. No.	Drug	Concentration (µg/ml)	Absorbance A	Mean* ± SD	% RSD
1	ATV	10	1.055	1.091 ± 0.00346	1.11
		10	1.088		
		10	1.094		
		10	1.101		
		10	1.102		
		10	1.106		
2	ASP	30	0.426	0.445 ± 0.00234	0.34

		30	0.431		
		30	0.439		
		30	0.441		
		30	0.446		
		30	0.449		

*Mean of three determinations

Table 3: Accuracy data for drug substance

S. No.	Drug	Concentration (µg/ml)	Absorbance			Mean*±SD	% RSD
			Set 1	Set 2	set 3		
1	ATV	8	1.432	1.456	1.146	1.344 ± 0.00234	0.87
		10	1.875	1.891	1.987	1.917 ± 0.00439	0.17
		12	2.036	2.218	2.23	2.154 ± 0.00762	0.91
2	ASP	48	0.564	0.594	0.599	0.585 ± 0.0092	0.76
		60	0.731	0.743	0.756	0.743 ± 0.00769	0.51
		72	0.801	0.834	0.877	0.837 ± 0.00865	1.09

*Mean of three determinations

Table 4: Recovery studies data of drug product

Drug	Conc. of sample µg/ml	Conc. of standard µg/ml	Recovered concentration µg/ml	% Recovery
ATV	8	10	17.1	95.1
	10	10	19.3	96.5
	12	10	21.7	98.6
ASP	60	75	134	99.2
	75	75	149	99.3
	90	75	163	98.7

Assay of fixed dose combination capsules (Table 5)

The developed and validated method was applied for the determination of atorvastatin calcium and aspirin in marketed formulation (capsules) AZTOR ASP manufactured by Sun Pharmaceuticals Ltd, containing 10 mg of atorvastatin calcium and 75 mg of aspirin.

Table 5: Assay values of atorvastatin calcium and aspirin

Brand name	Drugs	Label claim (mg)	Assay (%)
AZTOR ASP	ATV	10	94
	ASP	75	97.33

CONCLUSION

Both atorvastatin calcium and aspirin have shown similar spectra which cannot be resolved in absorbance mode for their simultaneous analysis. Increased resolution and differentiation of spectra was observed in derivative mode. The developed derivative spectrophotometric method can be employed for routine analysis of atorvastatin calcium and aspirin in marketed formulations. Fixed dose combined capsules were analysed using the developed method and assay results were within the limits for atorvastatin calcium and aspirin.

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