Simultaneous Estimation of Atorvastatin and Aspirin in Bulk and Capsule Dosage Form by Chemometric Assisted Spectrophotometric Methods

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ABSTRACT

Objective: To develop the UV-spectrophotometric method and to apply the Chemometric designs to the developed method for the simultaneous estimation of Atorvastatin calcium (ATR) and Aspirin (ASP) in intact capsule dosage form without further extraction. **Methods:** The UV-Spectrophotometric method was developed by using methanol as solvent for both the drugs and the data generated from the absorption spectra was mined by two Chemometric designs which were based on the principles of linear regression analysis method (LRC) and Crammer's matrix method (CRM). The wavelengths selected for linear regression analysis and crammers'matrix methods were 245 nm (wavelength of maximum absorption; λ_{max} of ATR) and 275 nm (wavelength of maximum absorption; λ_{max} of ASP). **Results:** Both the methods hold good linearity for ATR from 4-20 µg/ml and for ASP from 20-120 µg/ml with regression coefficient values of 0.9999 and 0.9991 respectively. The intraday and inter-day precision was found to be less than 2% RSD. The percentage recovery was in the range of 100.1-102.65 for Atorvastatin calcium and 99.95-101.15 for Aspirin by both the methods. The percentage assay was found to be 102.52 for ATR and 98.9 for ASP

by LRC method and 101.62 for ATR and 98.84 for ASP by CRM method. **Conclusion:** The developed methods neither require any cumbersome separation procedure nor complex derivatization procedures for the analysis of the two drugs and moreover they are effective in minimizing the errors in analysis, simple and economical.

Key words: Chemometrics, Regression analysis, Cramer's Matrix method, Atorvastatin, Aspirin.

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INTRODUCTION

Chemometrics is a branch of science that derives data by the application of mathematical and statistical methods, for the extraction of useful information from physical and chemical phenomena involved in a manufacturing process.[1-8] Chemometrics is used for multivariate data collection and analysis protocols, calibration, process modelling, pattern recognition and classification, signal correction and compression, and statistical process control. In view of the significant problems in the analysis of intricate multicomponent formulations by the conventional analytical methods in UV-spectroscopy and HPLC, Chemometric assisted analytical methods are designed to perform analytical investigation of such complex formulations.

Atorvastatin is chemically known as 7-[2-(4-fluorophenyl)-3-phenyl-4-(phenyl Carbamoyl) -5- (propan-2-yl)-1H-pyrrol-1-yl] -3, 5-dihydroxyheptanoate.[9] It is a selective competitive HMG-CoA reductase inhibitor. It reduces the risk of cardiovascular diseases. The Chemical structure of Atorvastatin was shown in Figure 1.

Aspirin is chemically known as 2-(acetyloxy) benzoic acid.[10] Acetylsalicylic acid is an analgesic, antipyretic, anti-rheumatic, and anti- inflammatory agent. Acetylsalicylic acid's mode of action as an anti- inflammatory and anti-rheumatic agent may be due to inhibition of synthesis and release of prostaglandins. The Chemical structure of Aspirin was shown in Figure 2.

The combination of Atorvastatin and Aspirin is used widely in the treatment of cardiovascular disorders and to lower the cholesterol levels.

Literature survey revealed that very few analytical methods like UV-Spectroscopy and HPLC methods were reported and no chemometric method was reported for the analysis of the above said combination.[11-23] Moreover, most of the proposed methods demand complex extraction

procedures for the separation of drugs in to individual components from the dosage form Thus the present study aims to design chemometric assisted spectroscopic methods for the intricate analysis of Atorvastatin and Aspirin without prior separation.

MATERIALS AND METHODS

Instruments used

Analytical Balance (Denver, M-220D), UV - Visible Spectrophotometer (Shimadzu-UV 1800), Ultra sonicator (PCI Analytics Ltd.-6.5L) were used in present study.

Data Handling Systems

UV-Probe 2.3.4 was used for the handling of UV -Visible Spectrophotometer. Algebrator (Ver. 5.0) and Easy Matrix Calculator Pro (Ver. 5.4) were used for resolving the data matrices.

Materials Used

The working standards of Atorvastatin and Aspirin were procured from Vance & Health, Pharmaceuticals, Pvt. Ltd, Hyderabad. Commercial formulation of the drugs were purchased from local market. Methanol AR-grade was procured from E. Merck (India) Ltd., Mumbai. Double distilled water was obtained from in-house distillation unit.

Preparation of Solutions

Preparation of Atorvastatin standard solutions

25 mg of Atorvastatin standard was weighed accurately and transferred to a 25 ml standard flask. The standard sample was dissolved using 15 ml
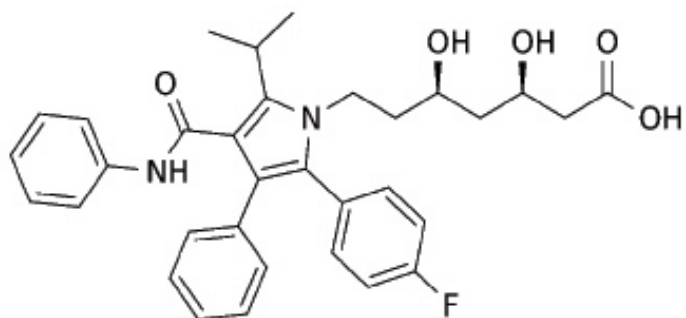
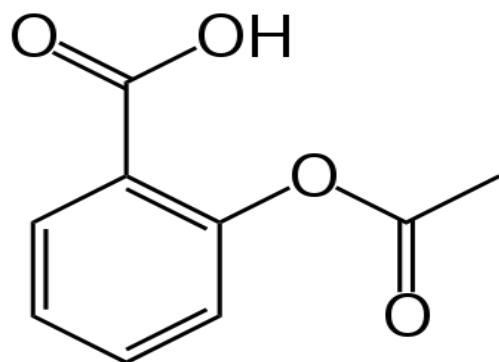**Figure 1:** Chemical Structure of Atorvastatin.

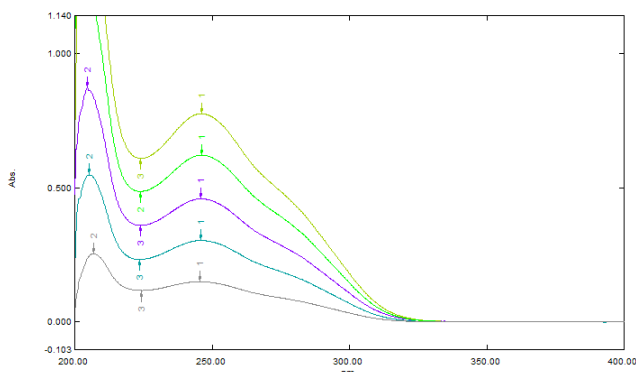

Figure 2: Chemical Structure of Aspirin.

Figure 3: Calibration curve of Atorvastatin (4-20 µg/ml) at 245 nm.

of methanol and made up to the mark with methanol. Further dilutions were made with the methanol to get the required concentrations of 4, 8, 12, 16 and 20 µg/ml.

Preparation of Aspirin standard solutions

10 mg of Aspirin standard was weighed accurately and transferred to a 10 ml standard flask. The standard sample was dissolved using 5 ml of methanol and made up to the mark with methanol. Further dilutions were made with the methanol to get the required concentrations of 20, 40, 60, 80,100 and 120 µg/ml.

Preparation of Atorvastatin and Aspirin sample solution

20 capsules were accurately weighed and triturated. Required quantity of powder equivalent to 10 mg of Atorvastatin and 75 mg of Aspirin was
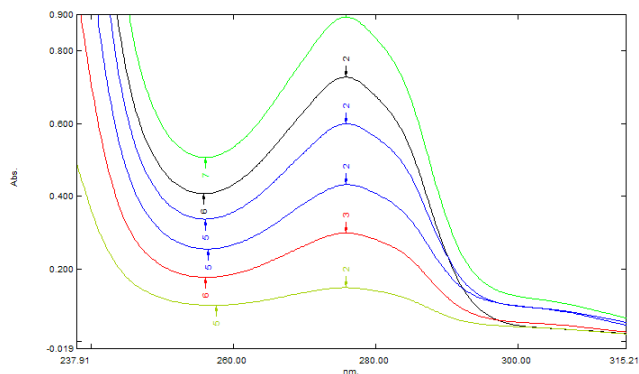

Figure 4: Calibration curve of Aspirin (20-120 µg/ml) at 275 nm.

weighed and transferred into a 10 ml volumetric flask. Required quantity of this stock solution was pipetted into volumetric flask to get 10 µg/ml, 75 µg/ml concentrations of Atorvastatin, Aspirin respectively.

Design of Chemometric Models

Two chemometric models were designed for the developed UV-spectro-photometric method for the simultaneous estimation of Atorvastatin and Aspirin. The first model was based on linear regression equation which includes variables like absorbance, slope and intercept of the calibration curve in the calculation of concentration of the drugs. Second model was based on the molar absorptivity values of the drugs.

Linear Regression Component (LRC) method

For LRC method, two wavelengths were considered for the analysis of the component mixture [ATR (X), and ASP (Y)]. The two linear regression equations were obtained by using the absorbance measured at two wavelengths against the concentrations of standard solution for each component. The slope values obtained from the linear regression analysis for each component were used for the formation of the matrix set. The wavelengths selected for the analysis were 245 nm (λ_{max} of ATR), 275 nm (λ_{max} of ASP).

Equations for the formation of matrix are:

$$A_{mix1} = b_{x1}C_x + b_{y1}C_y + a_{xy1}$$

$$A_{mix2} = b_{x2}C_x + b_{y2}C_y + a_{xy2}$$

Where, A_{mix1}, A_{mix2}, are the absorbances of the mixture of X, Y analytes at two wavelengths set. a_{xy1}, a_{xy2}, are the sum of intercepts of the linear regression equation at the two wavelengths. C_x and C_y are the concentrations of Atorvastatin and Aspirin. b_{x1}, b_{x2}, b_{y1} and b_{y2} are the slopes of Atorvastatin and Aspirin at 245 nm and 275 nm respectively.

Conversion of equation into matrix form:

$$\begin{bmatrix} Amix1- & axy1 \\ Amix2- & axy2 \end{bmatrix} = \begin{bmatrix} bx1 & by1 \\ bx2 & by2 \end{bmatrix} \times \begin{bmatrix} Cx \\ Cy \end{bmatrix}$$

Cramer's Matrix Method (CRM)

Molar absorptivity (ε) values were calculated by using the absorbance measured at 245nm, and 275 nm for each compound in the binarymixture. The selected wavelength values were λ_{max} of ATR, and ASP respectively. By using absorptivity (ε) values, a system of equations with two unknowns in the double mixture have been written as follows:

$$A_{m245} = \varepsilon_{AT,245}C_{ATR} + \varepsilon_{ASP,245}C_{ASP}$$

$$A_{m275} = \varepsilon_{ATR,275}C_{ATR} + \varepsilon_{ASP,275}C_{ASP}$$

Where A_m denotes the absorbance of the binary mixture and ε represents the values of molar absorptivity for the calculated ATR and ASP respectively at 245 nm & 275 nm. C is the molar concentration of ATR and ASP. The matrix simplifies and solves the system of equations with two unknowns as follows:

$$\begin{bmatrix} Am245 \\ Am275 \end{bmatrix} = \begin{bmatrix} \varepsilon\,ATR,245 & \varepsilon\,ASP,245 \\ \varepsilon\,ATR,275 & \varepsilon\,ASP,275 \end{bmatrix} x \begin{bmatrix} CATR \\ CASP \end{bmatrix}$$

$$\Delta = \begin{bmatrix} \varepsilon\,ATR,245 & \varepsilon\,ASP,245 \\ \varepsilon\,ATR,275 & \varepsilon\,ASP,275 \end{bmatrix}$$

$$\Delta_1 = \begin{bmatrix} Am245 & \varepsilon\,ASP,245 \\ Am,275 & \varepsilon\,ASP,275 \end{bmatrix}$$

$$\Delta_2 = \begin{bmatrix} \varepsilon\,ATR,245 & Am245 \\ \varepsilon\,ATR,275 & Am275 \end{bmatrix}$$

By applying Cramer's matrix rule the concentration of ATR, EZT and FNF can be found by

$$C_{ART} = \Delta_1/\Delta$$
$$C_{ASP} = \Delta_2/\Delta$$

Validation of the Spectrophotometric method[17,18]
Linearity and Range

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample. The range of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity.

For LRC and CRM, the absorbances were linear over the range of 4 µg/ml to 20 µg/ml for ATR and over the range of 20 µg/ml to 120 µg/ml for ASP at 245 nm and 275 nm respectively. The average absorbance of each concentration obtained was plotted against the concentration of the analytes.

Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

Intra-day precision was determined by analysing ATR and ASP for 3 times in the same day and Inter-day precision (ruggedness) was determined by analysing next 3 days at 245 nm, and 275 nm respectively for LRC and CRM.

Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. The accuracy of the method was determined by adding known quantities of the analyte (pure drug substances) to the drug product and applying the developed methods to determine the quantity of the drug present in the spiked sample.

Samples were spiked with 50, 100 and 150% level solutions of the standard and analysed. The experiment was performed in triplicate (n=3). Percent recovery values were reported.

$$\text{Accuracy} = \frac{\text{Amount of Sample Conc. found-Amount of Test Conc. taken}}{\text{Amount of Standard Conc. added}} \times 100$$

Assay

The commercial marketed formulation containing 10 mg of Atorvastatin and 75 mg of Aspirin was as the sample for the assay. The sample solution were treated in the same manner as the standard solution. The resulting solution was scanned under UV using methanol as blank.

$$\text{Percent Assay} = \frac{\text{Calculated qty of test sample (mg)}}{\text{Weight of test sample (mg)}} \times 100$$

RESULTS AND DISCUSSION

UV-Spectrophotometric method development

Different Solvents like Water, Methanol, 0.1N Hydrochloric acid and 0.1N Sodium hydroxide were employed for the optimization of the method. Methanol gave a single distinct peak with good absorbance for both the drugs i.e., ATR and ASP. So, it was employed as the solvent. From trial and error method, the λ_{max} of ATR and ASP bulk drugs were determined in methanol by UV spectrophotometer and absorption maximum was found at 245 nm and 275 nm respectively. The calibration curves of the drugs were presented in Figure 3 and 4. The abosrbances of the drugs at respective wavelengths were presented in Table 1.

Linear Regression Analysis (LRC)

The data obtained from the UV-spectrophotometric method were employed in this Chemometric design.

Conversion of equation into matrix form:

$$\begin{bmatrix} Amix1 - axyz1 \\ Amix2 - axyz2 \end{bmatrix} = \begin{bmatrix} bx1 & by1 \\ bx2 & by2 \end{bmatrix} \times \begin{bmatrix} Cx \\ Cy \end{bmatrix}$$

$$\begin{bmatrix} 0.987 - 0.0359 \\ 0.808 - 0.0035 \end{bmatrix} = \begin{bmatrix} 0.0392 - 0.0074 \\ 0.0249 - 0.0074 \end{bmatrix} \times \begin{bmatrix} Cx \\ Cy \end{bmatrix}$$

$$\begin{bmatrix} 0.9511 \\ 0.8045 \end{bmatrix} = \begin{bmatrix} 0.0392 - 0.0074 \\ 0.0249 - 0.0074 \end{bmatrix} \times \begin{bmatrix} Cx \\ Cy \end{bmatrix}$$

$$\begin{bmatrix} Cx \\ Cy \end{bmatrix} = \begin{bmatrix} 0.0392 - 0.0074 \\ 0.0249 - 0.0074 \end{bmatrix} \times \begin{bmatrix} 0.9511 \\ 0.8045 \end{bmatrix}$$

$$\begin{bmatrix} Cx \\ Cy \end{bmatrix} = \begin{bmatrix} 69.93 - 69.93 \\ -235.305 - 370.44 \end{bmatrix} \times \begin{bmatrix} 0.9511 \\ 0.8045 \end{bmatrix}$$

$$\begin{bmatrix} Cx \\ Cy \end{bmatrix} = \begin{bmatrix} 10.252 \\ 74.22 \end{bmatrix}$$

By applying linear regression method, the concentration of ATR and ASP was found to be 10.252 µg/ml and 74.22 µg/ml. The concentration of Atorvastatin (C_x) and Aspirin (C_y) present in the given formulation sample were found to be 10.252 µg/ml, and 74.22 µg/ml respectively.

Table 1: Linearity data of Atorvastatin and Aspirin

Atorvastatin			Aspirin		
Conc. (µg/ml)	Abs at 245 nm	Abs at 275 nm	Conc. (µg/ml)	Abs at 245 nm	Abs at 275 nm
04	0.149	0.090	20	0.176	0.149
08	0.303	0.187	40	0.321	0.299
12	0.458	0.286	60	0.470	0.431
16	0.620	0.392	80	0.622	0.597
20	0.774	0.486	100	0.735	0.726
			120	0.928	0.890

Table 2: Linearity and Range of Atorvastatin and Aspirin

Linear equation parameters	For LRC method		For Cramer's matrix method	
	ATR at 245 nm	ASP at 275 nm	ATR at 245 nm	ASP at 275 nm
Linear equation	y = 0.0392x +0.0093	y = 0.0074x+ 0.0001	y = 0.0392x +0.0093	y = 0.0074x+ 0.0001
R^2	0.9999	0.9991	0.9999	0.9991
Range	4-20 µg/ml	20-120 µg/ml	4-20 µg/ml	20-120 µg/ml
Acceptance criteria for R^2 value			Not less than 0.999	

Table 3: Precision data of Atorvastatin and Aspirin

Sample	Concentration (µg/mL)	Intra-day precision (n=3)	Inter-day precision (n=3)
		% RSD	% RSD
ATR at 245nm	8	1.84	1.21
	12	1.26	1.52
	16	1.13	0.72
ASP at 275nm	40	1.00	0.84
	60	0.69	0.81
	80	0.67	0.98
Acceptance criteria		% R.S.D<2.0	

Table 4: Accuracy data of Atorvastatin and Aspirin by LRC and CRM methods

Drug	Percentage	For LRC method	For CRM method
		% recovery*	% recovery*
ATR	50%	100.10	102.5
	100%	101.81	102.65
	150%	101.12	100.33
ASP	50%	101.15	101.15
	100%	99.95	99.95
	150%	100.9	100.9
Acceptance Criteria		Between 98.0 and 102.0%	

Table 5: Percentage Assay data of Atorvastatin and Aspirin by LRC method

| Drug	For LRC method	For CRM method
	% Assay	% Assay
ATR	102.52	101.62
ASP	98.9	98.84
Acceptance criteria	95- 105 %(w/w)	

Cramer's matrix method (CRM)

By substituting the values in matrix and solved and each compound was determined by solving the following operations (Δ = Determinant value of matrix)

$$\Delta = \begin{bmatrix} 0.0381 & 0.0077 \\ 0.0238 & 0.007462 \end{bmatrix}$$

$$\Delta_1 = \begin{bmatrix} 0.987 & 0.0077 \\ 0.808 & 0.007462 \end{bmatrix}$$

$$\Delta_2 = \begin{bmatrix} 0.0381 & 0.987 \\ 0.0238 & 0.808 \end{bmatrix}$$

By applying Cramer's matrix rule the concentration of ATR and ASP were found as follows

$$C_{ATR} = \Delta_1/\Delta = 0.001084/0.00010 = 10.8 \ \mu g/mL$$
$$C_{ASP} = \Delta_2/\Delta = 0.00729/0.00010 = 72.9 \ \mu g/mL$$

The concentration of Atorvastatin and Aspirin present in the given formulation sample were found to be 10.8µg/ml, and 72.9 µg/ml respectively.

Method Validation Parameters

The proposed spectrophotometric method was found to be linear and the data is presented in the Table 2. The linearity curves were shown in Graph 1and 2. The intra-day and inter-day precision values for both the chemometric designs were presented in Table 3. Accuracy was performed in terms of the Percent recovery values and the values for Atorvastatin and Aspirin by both the chemometric designs were presented in Table 4. The assay of the commercial formulation of the drugs were performed and their percentage assay values were presented in Table 5.
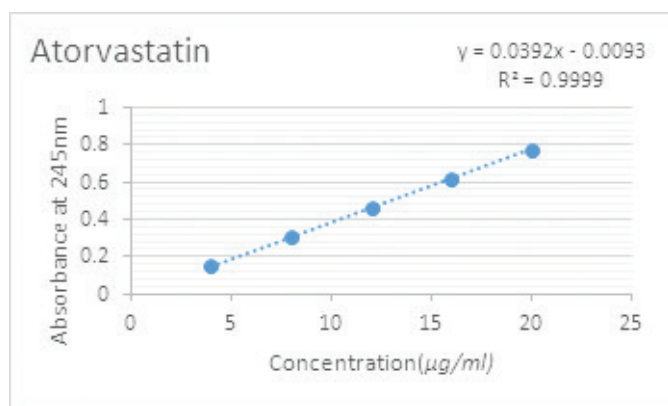
Graph 1: Linearity curve of Atorvastatin
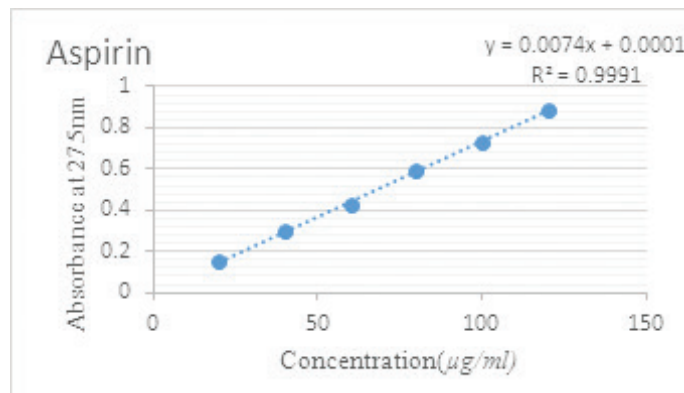
Graph 2: Linearity curve of Aspirin

CONCLUSION

Two simple, accurate, precise, economical methods were developed and validated to estimate Atorvastatin and Aspirin in bulk and Capsule dosage form. The developed methods were simple, economical, statistically evaluated and can be utilized for routine analysis in quality control laboratories. Application of Chemometric techniques helps in complete investigation of data present in the entire spectra for accurate estimation and minimization of error.

ACKNOWLEDGEMENT

CONFLICT OF INTEREST

This is a non-funding research work. There were no conflicts of interest.

ABBREVIATIONS USED

AR: Analytical Reagent; **ASP:** Aspirin; **ATR:** Atorvastatin; **Conc.:** Concentration; **CRM:** Crammer's Matrix Method; **HMG CoA-3:** Hydroxy-3-Methyl-Glutartyl-Co-Enzyme A; **HPLC:** High Performance Liquid Chromatography; **LRC:** Linear Regression Component; R^2: Correlation Coefficient; **RSD/R.S.D:** Relative Standard Deviation; **UV:** Ultra-Violet; λ_{max} : Wavelength of Maximum Absorbance.

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