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Chemometric Simultaneous Determination of Atorvastatin and Amlodipine in Bulk and Tablets

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Abstract: A simple, accurate and precise UV-spectrophotometric method based inverse least-squares was developed for the simultaneous determination of atorvastatin and amlodipine in tablet formulation. The absorbance values of the two analytes were linear with the concentration at the wavelengths taken at 5 nm interval over the range of 230 -260 nm. The calibration equations were developed using the absorbance values of nine synthetic mixtures containing different concentrations of two analytes measured at 5 nm intervalsin the range of 230 -260 nm. The developed equations werethen validated by calculating the analytes recovery from the analysis of a set of another five synthetic mixtures, the mean% recoveries were 100.02% and 100.06% with the corresponding% RSD of ±0.36 and±0.51 for atorvastatin and amlodipine, respectively. The calibration equations obtained were then used to obtain the concentration of each analyte in commercial samples. The mean % recoveries were 100.43% and 100.28% with the corresponding% RSD of ±0.78 and±0.85 for atorvastatin and amlodipine, respectively. The validity of the proposed method was confirmed through the statistical comparison of the obtained results with those obtained by a reference method utilizing high performance liquid chromatography for the determination of the two actives, the calculated t-values at (P=0.05, n =6) were 1.47 and 0.73 compared to the tabulated value of 2.23.

Keywords: Chemometric, Determination, Atorvastatin, Amlodipine

1. Introduction

Amlodipine besylate (AML) chemically is 3-ethyl-5-methyl (4RS0-2-[(2-aminoethoxy)methyl]-4- (2-chlorophenyl)-6-methyl-1,4–dihydropyridine-3,5-dicarboxylate benzenesulphonate. It is used is used in the management of hypertension and angina pectoris, it exerts its effect by blocking calcium ions transmembrane influx into vascular smooth muscles and cardiac smooth muscles [1].

Chemically Atorvastatin calcium (AVS) is [R- (R, R*)]-2-(4-fluorophenyl)-β-δ-dihydroxy- 5 (1- methylethyl)-3-phenyl-4 [(phenylamino) carbonyl] - 1H-pyrrole-1-heptanoic acid, calcium salt (2:1) trihydrate. It is a lipid lowering agent which inhibits the conversion of HMG-CoA to mevalonic acid a rate limiting step in hepatic cholesterol production. It is also used for primary and secondary prophylaxis of cardiovascular events [2].

The combination of AML and AVS as antihypertensive and lipid-lowering medications clinically used to reduce the risk of coronary artery disease, stroke and death in patients with cardiovascular risk factors [3].

Determination of multicomponent pharmaceutical dosage forms presents special challenge to the analytical chemists, as in most cases the spectral band(s) of one component overlaps with that of the other(s). Different approaches involving mathematical manipulation of the spectral data have been developed to resolve the overlapping bands. The approach followed principally varies with the extent of overlapping and the number of components involved [4-5].

The simultaneous determination of amlodipine besylate and atorvastatin calcium combination as tablets dosage form is not yet official in any compendia, however literature survey revealed that there are several reported methods; using analytical techniques such as chromatography, spectrophotometry, spectrofluorimetry, electrochemistry and chemometrics for the simultaneous determination of AML and AVS in binary mixtures [6-30].

The overlain absorbance spectra of AML and AVS (Figure

1) showed considerable overlapping in the range of 230 - 290 nm, hence application of the classical spectrophtometric techniques for the determination of their concentration in combined dosage forms is not possible. It is of interest to apply the inverse least-squares method (ILS) using UV-spectrophotometry for the simultaneous determination of AML and AVS combination in tablet dosage form. The success of the attempt is rewarding as it means that a simple, inexpensive, and reliable UV-spectrophotometric method can be used in place of expensive techniques based on separation for their determination.

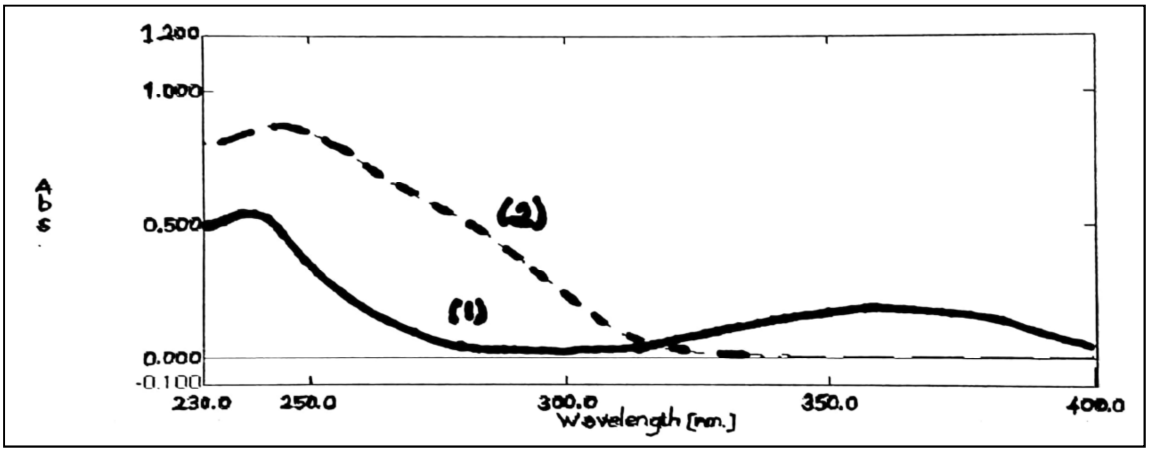

Figure 1. *Overlay absorbance spectra of (1) amlodipine besylate (7.5 µg/ml) and (2) atorvastatin calcium (20 µg/ml) in 50% aqueous methanol.*

Theoretical Background

Inverse least-squares (ILS), sometimes also known as P-matrix calibration, because, originally, it involved the application of multiple linear regression (MLR) to the inverse expression of the Beer-Lambert Law ofspectroscopy [31]:

$$C = P\,A \qquad (1)$$

where: concentration C, is a function of absorbance, A. This is the inverse of classical method where (A) is afunction of (C).

For the binary mixture, two concentration variables are involved, namely C1 and C2. The application of inverse least-squarestechnique generally executed in the following order:

Computation of the Calibration

Several standard solutions are prepared containing known varying concentrations of the pure the components under analysis (two components in this work). The absorbance of each of these solutions is measured at λ1, λ 2, λ 3, λ 4, λ 5 etc... to give correspondingly A1, A2, A3, A4, A5 etc... (The number of wavelengths selected should be greater than the number of components).

The absorbance data obtained are processed by suitable software program such as Minitab 16 to give two calibration equations. The calibration equations (Eq. 2 and Eq. 3) are worked out by multiple linear regressions based on the principles of least-squares.

$$C_1 = k_1 + \alpha_1 A_1 + \alpha_2 A_2 + \alpha_3 A_3 + \alpha_4 A_4 + \alpha_5 A_5 \text{ etc} \qquad (2)$$

$$C_2 = k_2 + \beta_1 A_1 + \beta_2 A_2 + \beta_3 A_3 + \beta_4 A_4 + \beta_5 A_5 \text{ etc} \qquad (3)$$

C1 and C 2 are concentrations of the components of the binary mixture, k_1 and k_2 are constants, α_i and β_i are coefficients. The constants and coefficients are worked outby the software program used.

Validation of the calibration equation

Another set of mixtures with known concentration of the components under analysis is prepared in the same manner as in (I) above andthe absorbance values are measured at the same wavelengths used in the calibration process. The measured absorbance values are then substituted in the calibration equations (2 and 3) togive C_1 and C_2.

The validity of the calibration equations is confirmed by obtaining by good recovery and low percent relative standard deviation values < 2% for the components assayed.

Samples analysis

Similarly, solutions of the samples are treated by measuring the absorbances, A_1, A_2, A_3, A_4, A_5 etc...at thecorresponding wavelengths λ_1, λ_2, λ_3, λ_4, λ_5 etc... The measured absorbance values of the samples arethen substituted in the calibration equations (2 and 3) togive C_1 and C_2.

2. Materials and Methods

2.1. Instrument

A double beam UV/Vis spectrophotometer, Shimadzu UV-1800, was employed with a matching pair of 1 cm quartz cells for all analytical work.

2.2. Chemicals and Reagents

Amlodipine besylate and atorvastatin calcium working standards were provided as a gift by Amipaharma Pharmaceuticals Industry-Sudan. Methanol of analytical grade and double distilled water were used throughout the analysis. Methanol 50%v/v in water was used as a diluent.

2.3. Commercial Formulation

Lorvast plus® tablets manufactured by Tabuk

Pharmaceuticals, Sudan, labeled to contain 20 mg of Atorvastatin as calcium and 5 mg of Amlodipine as besylate were purchased from the local market.

2.4. Preparation of Stock Standard Solutions

Usingthe diluents to give final concentration of amlodipine and atorvastatin 54µg/ml andAccurately weighed 7.5 mg of amlodipine besylate (equivalent to 5.4 mg amlodipine) and 35 mg atorovastatin calcium (equivalent to 32.3 mg atorvastatin) working standards were transferred into two separate 100 ml volumetric flask, dissolved using methanol and completed to mark 323.3µg/ml respectively.

2.5. Calibration Curves

Aliquot volumes (1- 5 ml) of each stock solution were transferred into two separate sets of five different 50 ml volumetric flasks and made to mark with the diluents, to give concentration of amlodipine and atorvastatin 1.08-5.4 µg/ml and 6.5-32.3µg/ml respectively. The absorbances of the calibration sets weremeasured over the range230-260 nmin 5 nm intervals andthelinear regression parameters were obtained for the absorbance values against their corresponding concentrations.

2.6. Preparation of the Calibration Mixtures

Nine laboratory prepared mixtures containing different concentrations of amlodipine and atorvastatin; covering the expected analytes concentration in the sample were prepared by mixing different volumes from the stock standard solutions of the two analytes in nine separate 50ml volumetric flasks, the volumes of the flaks were made to markwith the diluent.

2.7. Preparation of Validation Mixtures

Five laboratory prepared mixtures containing different concentrations of amlodipine and atorvastatin were prepared by mixing different volumes of the stock standard solutions in five separate 50ml volumetric flasks, the volumes of the flaks were made to markwith the diluent. The absorbance of the mixtures was measured over the range230-260 nm in5 nm intervals and used to determine their corresponding concentrations by substituting the measured values in the calibration equations.

2.8. Sample Preparation

Twenty tablets were weighed and finely powdered in a mortar, and the average weight of tablet was calculated. An amount ofpowder equivalent to one tablet was accurately weighed, transferred into a100ml volumetric flask and dissolved in methanol with sonication for 15 minutes and completed to the mark with the dilutent. The solution was then filtered using 0.45µm nylon filter; 4 ml of the filtrate were diluted to 50 ml with the dilutent. The absorbance of the sample solution was measured over the range230-260 nm in5 nm intervals and used to calculate the concentration of each analyte by direct substitution in the calibration equations.

3. Results and Discussion

Linear regression analysis of the absorbance values of amlodipine and atorvastatin over the range of 230 -260 nm in5 nm intervals against their corresponding concentrations were performed. The two analytes showed good correlation between the absorbance and concentration (r^2 >0.99). The regression analysis data of the two analytes are shown in Table 1.

Table 1. *Analytes regression analysis data.*

Wavelength (nm)	Regression analysis equation (y =bx+a)	
	Amlodipine	**Atorvastatin**
230	y=0.0617+0.0190 (r^2 =0.9982)	y=0.0351x+0.0115 (r^2 =0.9984)
235	y=0.068+0.0309 (r^2 =0.9982)	y=0.0388-0.0066 (r^2 =0.9996)
240	y=0.0673x+0.0047 (r^2 =0.9996)	y=0.0423x-0.0047 (r^2 =0.9997)
245	y=0.0574x-0.0051 (r^2 =0.9999)	y=0.0437x+0.0137 (r^2 =0.9979)
250	y=0.0413x -0.0089 (r^2 =0.9982)	y=0.044x -0.0131 (r^2 =0.9988)
255	y=0.02801x+0.0049 (r^2 =0.9978)	y=0.0422x-0.009 (r^2 =0.9997)
260	y=0.0212x-0.0048 (r^2 =0.9979)	y=0.377x-0.0009 (r^2 =0.9999)

3.1. Computation of Calibration Equations

Table 2 displays the absorbance values of the nine synthetic mixtures used for computation of the constants and the coefficient of the calibration equations using Minitab 16 software.

The following calibration equations were obtained:

$$C1 = -1.38 + 28.57A230 - 0.0003A235 - 30.38A240 + 23.78A245 - 78.17A250 + 83.41A255 + 3.73A260 \quad (4)$$

$$R2 = 1.00$$

$$C2 = 0.049 - 8.16\,A230 - 0.0005\,A235 + 23.68\,A240 + 1.23\,A245 + 35.12\,A250 - 57.75\,A255 + 1.37\,A260 \quad (5)$$

$$R2 = 1.00$$

where

A_i are the absorbance values of the binary mixtures at the selected wavelengths, C1 and C2 stand for the concentrations of atorvastatin and amlodipine, respectively. The coefficient of determination, R2, indicated excellent linearity.

Table 2. *Absorbance data of the synthetic mixtures.*

Mix. No.	Concentration		Absorbance						
	AVS	AML	λ230	λ235	λ240	λ245	λ250	λ255	λ260
1	19.40	3.24	0.990	1.088	1.162	1.146	1.083	0.984	0.822
2	12.93	4.33	0.770	0.905	0.929	0.894	0.813	0.726	0.611
3	25.87	4.33	1.229	1.478	1.546	1.562	1.451	1.33	1.175
4	25.87	3.24	1.226	1.389	1.473	1.505	1.427	1.302	1.149
5	19.40	4.33	1.035	1.185	1.22	1.201	1.114	1.003	0.847
6	25.87	2.16	1.172	1.327	1.406	1.425	1.372	1.266	1.135
7	12.93	2.16	0.680	0.767	0.779	0.785	0.74	0.667	0.581
8	12.93	3.24	0.754	822	0.858	0.871	0.794	0.698	0.600
9	19.40	2.16	0.933	1.077	1.055	1.102	1.057	0.951	0.853

3.2. Checking the Validity of the Calibration Equations

The recovery and percent relative standard deviation (RSD%) and data obtained by application of the mixtures for AVS and AML by ILS have been summarized in Table 3. The statistical parameters of recovery percentagewere used to evaluate the percent relative standard deviation for theproposed models.

As shown in Table 3, values of RSD% and recovery for both compounds by ILS method indicate that the proposed method is suitable for successful determination of the two analytes in combination without any prior separation.

Table 3. *Model validation data (recovery from synthetic mixtures).*

Atorvastatin (µg/ml)	AVS recovered (µg/ml)	% Recovery	Amlodipine (µg/ml)	AML recovered (µg/ml)	% Recovery
19.33	19.26	99.66	3.23	3.22	99.58
12.94	12.94	100.02	4.33	4.33	100.01
25.80	25.73	99.73	4.31	4.30	99.66
26.02	26.17	100.58	3.28	3.31	100.97
19.42	19.44	100.11	4.33	4.33	100.10
Mean% recovery		100.02	Mean% recovery		100.06
RSD (%)		0.36	RSD (%)		0.51

3.3. Analysis of Commercial Sample

Table 4 exhibits the% recoveries obtained by applying the developed chemometric method to the simultaneous determination of atorvastatin and amlodipine in tablet dosage form. The mean% recoveries were 100.43% and 100.28% with the corresponding % RSD of ±0.78 and ±0.85 for atorvastatin and amlodipine, respectively.

Table 4. *Values of% recoveries of atorvastatin and amlodipine in tablet formulation.*

Atorvastatin (µg/ml)	AVS recovered (µg/ml)	% Recovery	Amlodipine (µg/ml)	AMLrecovered (µg/ml)	% Recovery
14.86	14.93	100.51	2.88	2.87	99.83
14.84	14.90	100.40	2.87	2.86	99.59
14.70	14.61	99.44	2.90	2.91	100.48
15.02	15.26	101.62	2.90	2.92	100.59
14.74	14.71	99.75	2.93	2.99	101.75
14.90	15.03	100.83	2.87	2.85	99.45
Mean% recovery		100.43	Mean% recovery		100.28
RSD%		0.78	RSD%		0.85

The validity of the method was further assessed by statistically comparing the results obtained with those of a reported high performance liquid chromatographic method [30]. No significant difference between the two methods observed at (P=0.05; n=6), accordingly the developed method can be considered as accurate and precise as the reported liquid chromatographic method (Table 5).

Table 5. *Results of the proposed method compared to the reference method [30].*

		% content ± sd	t – calculated (t - tabulated)
Proposed method	Amlodipine	100.28 (0.86)	
	Atorvastatin	100.43 (0.57)	1.47 (2.23)
Reference method	Amlodipine	100.96 (0.73)	0.73 (2.23)
	Atorvastatin	100.13 (0.63)	

4. Conclusions

A simple, accurate and precise UV-spectrophotometric method based on chemometrics was developed for the simultaneousdetermination of atorvastatin and amlodipine intablet formulation without prior separation. The cost effectiveness in term of time and moneyrenders the method as suitablealternative to otherexpensive techniques e.g. chromatographic methods for theanalysis of binary mixtures of compounds with overlappedspectra in laboratories and countries where such sophisticated equipments are not affordable. The accuracy and simplicity of the method suggest it suitability in caseswhere quick results are demanded e.g. as an in-process analysis procedure during blend analysis in industrial setups.

Conflict of Interest

The authors do not have any possible conflicts of interest.

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