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Simple spectrophotometric methods for the determination of amlodipine and atorvastatin in bulk and tablets

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Abstract

Background: Simultaneous spectrophotometric determination of samples containing more than one analyte presents analytical challenge; the choice of an analytical procedure is strictly related to the extent of overlapping between the individual absorption peaks of these components; if the absorption peaks are satisfactorily resolved, the determination is not problematic, but if the individual component signals are partly or totally overlapped, then powerful techniques are needed. Combined amlodipine and atorvastatin are typical example where special techniques are needed to resolve bands overlapping.

Results: Application of multiwavelength regression and absorbance factor methods to the analysis of atorvastatin and amlodipine combination proved to be satisfactorily capable of accurate and precise determination of the two analytes. The two methods recoveries were very close to the expected analytes concentrations, and the precision of the methods was < 2% relative standard deviation. Statistical comparison indicated that there is no significant difference between the assay results obtained by the two method as the calculated *t* values 0.91 and 1.13 for amlodipine and atorvastatin, respectively, were less than the tabulated *t* value 2.23 at 95% confidence level.

Conclusion: The proposed methods are accurate, precise, simple and inexpensive. They can be applied successfully to the analysis of the two drugs in combined dosage form.

Keywords: Multiwavelength regression, Absorbance factor, Atorvastatin, Amlodipine, Spectrophotometry

Background

Amlodipine (AML), chemically, is 3-O-Ethyl5-O-methyl 2-(2-aminoethoxymethyl)-4-(2-chloro-phenyl)-6-methyl-1,4-dihydropyridine-3,5 dicarboxylate (Fig. 1a). It has been used in the management of hypertension as it blocks calcium ions transmembrane influx into vascular smooth muscles and cardiac smooth muscles [1].

Atorvastatin (AVS), chemically, is [R-(R*,R*)]-2-(4-Fluorophenyl)-b,d-dihydroxy-5-(1-methyl-ethyl)-3-phenyl-4-[(phenylamino)carbonyl]-1H-pyrrole-1 heptanoic acid (Fig. 1b). It has been used as a lipid-lowering agent as it inhibits the conversion of HMG-CoA to mevalonic acid a rate limiting step in hepatic cholesterol production [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].

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 chemometry for the simultaneous determination of AML and AVS in binary mixtures [4–29].

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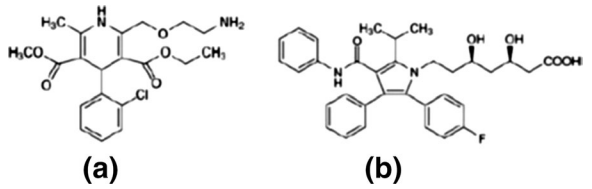

Fig. 1 Chemical structure of **a** amlodipine and **b** atorvastatin

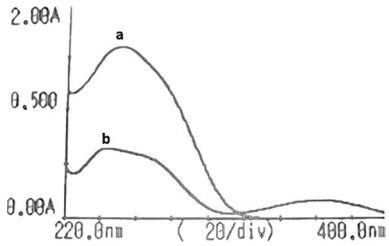

Fig. 2 Overlay absorbance spectra of **a** atorvastatin (20 µg/mL) and **b** amlodipine (7.5 µg/mL) in 50% aqueous methanol

Amlodipine and atorvastatin absorbance spectra (Fig. 2) showed extensive overlapping in the region of 220–300 nm, and resolution of these overlapping spectra and subsequent determination of the analytes concentrations in combined dosage forms is only possible if more sophisticated and expensive techniques are used. The objective of this work was to use multiwavelength regression analysis [30] and absorbance factor method [31] as simple, inexpensive, and reliable UV-spectrophotometric methods in place of expensive techniques based on separation or sophisticated methods using specialized computer programs.

Theoretical background

Multiwavelength regression analysis

Assuming additivity of absorbance and validity of Lambert–Beer's law, the absorbance of a mixture is the sum of the absorbance values of its individual components.

If we have a mixture consisting of two components, 1 and 2, with an unknown concentration of C_1 and C_2, then absorbance of the unknown mixture,

$$A_{\text{mixture}} = A_1 + A_2$$

applying Beer's law: $A_1 = \varepsilon_1 b C_1$ and $A_2 = \varepsilon_2 b C_2$.

Substituting:

$$A_{\text{mixture}} = \varepsilon_1 b C_1 + \varepsilon_2 b C_2.$$

However, the absorbencies of standard solutions of the same substances will follow the same Beer's law relationship and have the same molar absorbance, ε, and one centimeter path length, b, as the unknown solutions under the same conditions.

Therefore, we can write:

$$A_{\text{standard 1}} = \varepsilon_1 b C_{\text{standard 1}} \quad \text{and} \quad A_{\text{standard 2}} = \varepsilon_1 b C_{\text{standard 2}}$$

Rearranging these relationships:

$$\varepsilon_1 b = A_{\text{standard 1}} / C_{\text{standard 1}} \quad \text{and}$$
$$\varepsilon_2 b = A_{\text{standard 2}} / C_{\text{standard 2}}$$

Substituting,

$$A_{\text{mixture}} = A_{\text{standard 1}} / C_{\text{standard 1}} C_1 + A_{\text{standard 2}} + A_{\text{standard 2}} / C_{\text{standard}} . C_2$$

or

$$A_{\text{mixture}} = C_1 / C_{\text{standard 1}} . A_{\text{standard 1}} + C_2 / C_{\text{standard 2}} . A_{\text{standard 2}}$$

Dividing by $A_{\text{standard 1}}$ and simplifying we obtain:

$$A_{\text{mixture}} / C_{\text{standard 1}} = C_1 / C_{\text{standard 1}} + C_2 / C_{\text{standard 2}} . A_{\text{standard 2}} / A_{\text{standard 1}} \quad (1)$$

Therefore, a plot of

$$A_{\text{mixture}} / C_{\text{standard 1}} \quad \text{versus} \quad A_{\text{standard 2}} / A_{\text{standard 1}} \quad (2)$$

will give

$$\text{slope} = C_2 / C_{\text{standard 2}} \quad \text{and} \quad \text{intercept } C_1 / C_{\text{standard 1}}$$

That is, the concentration of the unknown component 2 (C_2) in the mixture equals the slope times the concentration of the standard solution for component 2. Likewise, the concentration of the unknown component 1 (C_1) in the mixture equals the product of the intercept times the concentration of the standard solution for component1 or simply

$$C_1 = \text{intercept} \times C_{\text{standard 1}} \quad (3)$$

and

$$C_2 = \text{slope} \times C_{\text{standard 2}} \quad (4)$$

Absorption factor method

The method depends on the fact that in the overlapping spectra of a mixture of two drugs, e.g., X and Y, X has some interference at $\lambda_{\max}$ of Y (λ_1) and has no absorption at another wavelength (λ_2) that Y show absorbance at λ_1; the absorbance of the mixture at (λ_2) equals the absorbance of Y.

$$\text{Absorbance of } Y \text{ at } \lambda_1 = (\text{abs } Y_{\lambda 1} / \text{abs } Y_{\lambda 2}) . \text{abs}_{\lambda 2}(X + Y) \quad (5)$$

$$\text{Absorbance of } X \text{ at } \lambda_1 = \text{abs}_{\lambda 2}(X+Y) - (\text{abs } Y_{\lambda 1}/\text{abs } Y_{\lambda 2}).\ \text{abs}_{\lambda 2}(X+Y) \quad (6)$$

The proposed methods allow accurate and precise determination of binary mixtures of compounds with highly overlapped spectra using simple and easy mathematics instead of more complex mathematical procedures.

Methods

Materials and reagents

Amlodipine besylate and atorvastatin calcium working standards were supplied by Amipharma Pharmaceutical Industries—Sudan and Methanol from Carlo Erba-Italy.

Lorvast plus tablets manufactured by Tabuk Pharmaceuticals-Sudan labeled to contain 20 mg of Atorvastatin as calcium and 5 mg of Amlodipine as besylate and were purchased from the local market.

The diluent (50% v/v aqueous methanol) was prepared by mixing equal volumes of methanol and distilled water.

Instrument and software

A double beam UV/Vis spectrophotometer, Shimadzu UV-1800, was employed with a matching pair of 1 cm quartz cells for all analytical work. Microsoft Excel Spreadsheet 2013 was used for data analysis.

Standards stock solutions

Accurately weighed about 15 mg Amlodipine besylate and 25 mg atorvastatin calcium working standard were separately weighed and transferred into separate 100 mL volumetric flasks, dissolved using the diluent and the volumes were completed to the mark using the diluent.

Calibration curves

Aliquot volumes (1–5 mL) from each stock solution were transferred into two separate sets of five different 50 mL volumetric flasks and made to mark with the diluent to give amlodipine in the range of 2–10 µg/mL and atorvastatin in the range of 4–20 µg/mL. The absorbance of these solutions was measured at the wavelengths selected for each method, and the linear regression parameters were obtained for the absorbance values against their corresponding concentrations.

Preparation of synthetic mixtures

Six laboratory synthetic mixtures containing different concentrations of amlodipine and atorvastatin were prepared by mixing different volumes from the two stock solutions in six separate 50 mL volumetric flask and making the volume to the mark with the diluent.

Preparation of analytical standards (multiwavelength regression method)

From each analyte, standard stock solution 5 mL were transferred into a separate 50 mL volumetric flasks and made to mark with the diluent.

Sample preparation

Twenty tablets were accurately weighed and crushed to a fine powder; the weight of powder equivalent to one tablet was transferred into a 100 mL volumetric flask, dissolved with the diluent, sonicated for 15 min and filtered using 0.45 µm nylon syringe filter; 5 mL of the filtrate were diluted to 50 ml using the diluent.

General procedure

Multiwavelength regression method

The absorbance of the synthetic mixtures, standards and samples were measured in the range of 230–260 nm at 5 nm intervals. For the application of the method, the ratio of the synthetic mixture or sample absorbances to that of atorvastatin analytical standard solution at each wavelength was calculated and plotted against the ratio of absorbance values obtained for amlodipine analytical standard solution to the same atorvastatin standard solution used in the first instance. The slope and intercept of the straight line obtained were then used to calculate the concentration of each analyte in the mixture or the sample.

Absorbance factor method

The absorbance factor was initially calculated from the absorbance values of amlodipine calibration series as the ratio of the absorbance of each solution at 244 nm to its corresponding absorbance at 365 nm. The average absorbance factor obtained was then used to correct the absorbance of atorvastation by removing the interference due amlodipine at 244 nm. The concentration of each analyte was calculated using its corresponding regression equation.

Results

Spectral characteristics

UV absorbance spectra of amlodipine 15 µg/mL and atorvastatin 23 µg/mL were recorded over the range of 200–400 nm against the diluent as a blank (Fig. 2). AML showed maximum absorbance at 244 nm and 365 nm while AVS showed maximum absorbance at

Table 1 Amlodipine linearity data (multiwavelength regression method)

Parameter	230 nm	235 nm	240 nm	245 nm	250 nm	255 nm	260 nm
b	0.0379	0.0447	0.0291	0.0131	0.0057	0.0023	0.0006
a	0.0067	0.0047	0.0018	0.0028	0.0021	0.0011	0.005
r	0.9994	0.9995	0.9994	0.9997	0.9998	0.9991	1.000
S_b	0.0009	0.0009	0.0007	0.0002	0.00009	0.00007	0.00003
S_a	0.0074	0.0076	0.0056	0.001	0.0007	0.0005	0.00006
LOD (µg/mL)	0.396	0.344	0.388	0.287	0.240	0.454	0.626
LOQ (µg/mL)	1.199	1.042	1.176	0.869	0.727	1.376	1.897

b slope, *a* intercept, *r* correlation coefficient, S_b standard deviation of the slope, S_a standard deviation of the intercept, *LOD* limit of detection, *LOQ* limit of quantitation

Table 2 Atorvastatin linearity data (multiwavelength regression method)

Parameter	230 nm	235 nm	240 nm	245 nm	250 nm	255 nm	260 nm
b	0.0125	0.0365	0.0365	0.0314	0.0275	0.0229	0.0171
a	0.0021	− 0.0024	0.0079	0.015	− 0.0086	0.0008	0.0109
r	0.9999	0.9998	0.9999	0.9998	0.9999	0.9998	0.9999
S_b	0.00007	0.00052	0.00038	0.00042	0.00034	0.00031	0.00021
S_a	0.0009	0.0068	0.00491	0.0056	0.0045	0.0042	0.0029
LOD (µg/mL)	0.236	0.595	0.436	0.571	0.523	0.579	0.536
LOQ (µg/mL)	0.717	1.804	1.321	1.730	1.585	1.755	1.623

b slope, *a* intercept, *r* correlation coefficient, S_b standard deviation of the slope, S_a standard deviation of the intercept, *LOD* limit of detection, *LOQ* limit of quantitation

244 nm and no absorbance at 365 nm; hence, AML can be determined by measuring its absorbance at 365 nm. Absorbance of AVS at 244 nm can be determined by use of absorbance factor.

For the application of multilinear regression method, absorbance measurements were taken at 10 nm intervals over the range of 230–260 nm.

Linearity

Calibration plots at the wavelength selected for each method were linear over the concentration range, $r^2 > 0.99$.

The limit of quantification (LOQ) and limit of detection (LOD) were evaluated from the regression data using the following equations:

$$\mathrm{LOQ} = 10 * \sigma / \mathrm{S}$$
$$\mathrm{LOD} = 3.3 * \sigma / \mathrm{S}$$

σ = the standard deviation of the response, S = the slope of the calibration curve.

The absorbance factor determined from the regression data was found to be 2.7042 which was then used to remove the interference of amlodipine with absorbance of atorvastatin at 244 nm. Tables 1, 2, 3, 4 summarize the results of regression analysis.

Table 3 Amlodipine linearity data (absorbance factor method)

Parameter	244 nm	365 nm
b	0.0457	0.0165
a	− 0.00031	0.0016
r	0.9999	0.9998
S_b	0.000294	0.00027
S_a	0.002104	0.00194
LOD (µg/ml)	0.147	0.376
LOQ (µg/ml)	0.446	1.138

b slope, *a* intercept, *r* correlation coefficient, S_b standard deviation of the slope, S_a standard deviation of the intercept, *LOD* limit of detection, *LOQ* limit of quantitation

Table 4 Atorvastatin linearity data (absorbance factor method)

Parameter	244 nm
b	0.0399
a	0.0046
r	0.9999
S_b	0.00018
S_a	0.002810
LOD (µg/ml)	0.2249
LOQ (µg/ml)	0.6815

b slope, *a* intercept, *r* correlation coefficient, S_b standard deviation of the slope, S_a standard deviation of the intercept, *LOD* limit of detection, *LOQ* limit of quantitation

Recovery

Multiwavelength regression method

Samples and standards absorbance measurements were taken several wavelengths. Linear plots were obtained from the ratios of absorbance the two drugs mixture (or the sample) to the absorbance of atorvastatin analytical standard solution versus the ratios of absorbance amlodipine analytical standard solution to that of the same atorvastatin standard analytical solution used earlier. The slopes and intercepts of the linear plot obtained were then used to calculate the concentration of each analyte. The recovery and percent relative standard deviation (RSD%) were obtained and are summarized in Table 5.

Absorbance factor method

The interference of amlodipine with the absorbance due to atorvastatin at 244 nm was removed using the absorbance factor. The concentration of each analyte was then calculated from its corresponding regression equation of absorbance versus concentration, at 244 nm for atorvastatin and 365 nm for amlodipine. The recovery and percent relative standard deviation (RSD%) were obtained and are summarized in Table 6.

Precision of the proposed methods

The precision of the proposed methods was studied by analyzing six independent replicate samples from the commercial product on two different days. The data are reported in Tables 7 and 8.

Commercial product analysis

The percent recoveries and relative standard deviations (%RSD) obtained by applying the proposed methods to the analysis of independent six replicate samples from the commercial product are reported in Tables 9.

Furthermore, statistical comparison indicated that there is no significant difference between the assay results obtained by the two method as the calculated t values

Table 5 Multiwavelength regression method recovery results

Mixture no.	Regression equation $Y=bx+a$	Amlodipine (μg/mL)		% Content	Atorvastatin (μg/mL)		% Content
		Theoretical	Actual		Theoretical	Actual	
1	$0.5987b+0.5881$ ($r=0.9999$)	13.01	12.94	99.46	6.49	6.36	98.00
2	$0.7960b+0.8059$ ($r=0.9998$)	17.33	17.21	99.31	8.65	8.72	100.81
3	$0.9807b+0.9904$ ($r=0.9998$)	21.60	20.77	98.15	10.82	10.72	99.08
4	$0.5915b+0.7944$ ($r=0.9999$)	13.00	12.78	98.31	8.65	8.60	99.42
5	$0.7920b+0.5932$ ($r=0.9997$)	17.30	17.12	98.96	6.49	6.42	98.92
6	$0.5944b+0.3943$ ($r=0.9998$)	13.00	12.84	98.77	4.33	4.27	98.61
Analytical standard		21.62 (μg/mL)			10.82 (μg/mL)		
Mean		98.83			99.14		
RSD (%)		0.53			0.96		

Table 6 Absorbance factor method recovery results

Mixture no.	Amlodipine (μg/ml)		% Content	Atorvastatin (μg/ml)		% Content
	Theoretical	Actual		Theoretical	Actual	
1	4.38	4.37	99.77	8.33	8.52	102.24
2	6.58	6.56	99.70	12.50	12.81	102.48
3	8.77	8.74	99.66	16.67	16.60	99.58
4	8.77	8.68	98.97	8.33	8.34	100.12
5	4.38	4.37	99.77	16.67	16.59	99.52
6	6.58	6.56	99.70	16.67	17.07	102.40
Mean		99.59		101.06		
RSD (%)		0.31		1.44		

Table 7 The precision of the multiwavelength regression method

AML (µg/mL)		% LC	AVS (µg/mL)		% LC
Actual	**Found**		**Claim**	**Found**	
Repeatability (n = 6)					
5.02	5.00	99.60	20.10	20.29	100.97
4.94	4.89	98.99	19.74	19.71	99.85
5.11	5.10	99.80	20.43	20.55	100.59
5.01	4.98	99.40	20.02	20.30	101.40
4.83	4.82	99.79	19.31	19.31	100.00
4.94	4.42	98.44	17.97	17.98	100.06
Mean		99.34	Mean		100.47
RSD%		0.55	RSD%		0.61
Intermediate precision (n = 6)					
5.00	5.04	100.80	20.00	20.30	101.55
5.00	4.98	99.50	20.00	20.38	101.90
5.00	5.04	100.84	20.00	19.97	99.90
4.99	4.94	98.92	19.96	20.18	101.10
5.00	5.03	100.60	19.97	19.94	98.85
5.00	4.93	98.68	20.00	20.36	101.85
Mean		99.87	Mean		101.03
RSD%		0.98	RSD%		0.94

Table 8 The precision of the absorbance factor method

AML (µg/mL)		% Content	AVS (µg/mL)		% Content
Actual	**Found**		**claim**	**Found**	
Repeatability (n = 6)					
4.99	4.92	98.50	19.99	19.89	99.96
4.99	4.92	98.50	19.99	19.89	99.96
4.99	4.92	98.50	19.99	19.86	99.83
5.00	4.97	98.49	20.00	19.83	99.13
5.00	4.91	99.30	20.00	19.89	99.47
4.99	4.91	98.50	19.99	17.87	99.84
Mean		98.63	Mean		99.70
RSD%		0.43	RSD%		0.34
Intermediate precision (n = 6)					
5.00	4.97	9.48	20.00	19.90	99.50
4.9	4.92	100.31	19.90	19.99	100.47
5.00	4.92	98.30	20.00	19.97	99.84
4.99	4.97	99.68	19.90	19.95	101.10
4.99	5.03	100.87	19.90	19.83	98.85
5.00	5.03	100.67	20.00	19.88	101.85
Mean		99.87	Mean		99.86
RSD%		0.98	RSD%		0.42

0.91 and 1.13 for amlodipine and atorvastatin, respectively, were less than the tabulated *t* value 2.23 at 95% confidence level, and the results are shown in Table 9.

Table 9 Analysis of the commercial product and their statistical comparison

Parameter	Multiwavelength regression method	Absorbance factor method
Amlodipine (n = 6)		
% LC (RSD%)	99.34 (0.55)	98.63 (0.43)
Atorvastatin (n = 6)		
% LC (RSD%)	100.47 (0.61)	99.70 (0.34)
t test		
Calculated *t* value	AML	0.91
	AVS	1.13
t tabulated		2.23

Discussion

The absorbance values of the calibration standards used for the two methods showed good proportionality with the concentrations, the squared correlation coefficients were > 0.99 and the calibration plots showed small insignificant intercepts in both cases [32].

Good agreement between the theoretical and the actual concentrations of the analytes in the synthetic mixtures was obtained with small relative standard deviation (RSD%) values (< 2%), confirming satisfactory recovery by the two methods.

The repeatability and intermediate precision of both methods produced relative standard deviation (RSD%) values < 2%. Adequate % recoveries and low % RSD were obtained when the two proposed method applied to the analysis of the commercial product.

Statistical analysis of the precision data applying student *t* test confirmed that there was no day to day variation in the results.

The two methods were found to satisfy the requirements of the International Conference on Harmonization (ICH) with regard to linearity, precision and accuracy [33].

Statistical comparison indicated that there is no significant difference between the assay results obtained by the two method as the calculated *t* values 0.91 and 1.13 for amlodipine and atorvastatin, respectively, were less than the tabulated *t* value 2.23 at 95% confidence level [34].

Conclusions

Simple, accurate, precise and cost effective spectrophotometric methods have been developed and validated for the determination of amlodipine and atorvastatin in combined dosage form. The proposed methods are also having the advantage of using very simple spectrophotometric approaches for the determination of the two drugs

mixture without prior separation or using sophisticated instruments and software. Either of the two methods can be used with confidence for the analysis of the two drugs mixture since no significant differences in the analytical results were observed between them. The proposed methods are beneficial for poor countries where acquisition of such equipment is not affordable.

Abbreviations

AML: Amlodipine; AVS: Atorvastatin; %RSD: Relative standard deviations; LOQ: Limit of quantification; LOD: Limit of detection; σ: The standard deviation of the response; S: The slope of the calibration curve; UV: Ultraviolet; ICH: International Conference on Harmonization of registration requirements.

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Authors' contributions

HF and SE performed the analysis, reported the results and prepared the first draft. The corresponding author revised the calculations and prepared the final manuscript. All authors have read and approved the manuscript.

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Availability of data and materials

All data and materials are available upon request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable as our study does not include patients.

Competing interests

The authors declare that they have no competing interests.

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