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Development of Absorption Factor Spectropotometric Method for the Simultaneous Determination of Amlodipine besylate and Atorvastatin calcium in Bulk and in Tablets

By

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B.Pharm (U of G) 2012

A Thesis Submitted in Partial Fulfillment of the Requirements for Master Degree in Drug Quality Control

Supervisor

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Dedication

To my parents, my brothers and sisters, my little nephews and my dear husband.

Acknowledgement

I wish to express my honest and sincere gratitude to my supervisor Dr. Imad Osman AbuReid, department of Pharmaceutical chemistry, Faculty of Pharmacy, National Ribat University. His motivation and encouragement were of a great value for this work to see light, for enlightening my mind with his valuable knowledge and guidance, and for his time.

I would like to thank the technical staff of the pharmaceutical chemistry department in National Ribat University.

My family and my beloved husband thank you for being there beside me and for helping me through every step of this work.

Abstract

Combination dosage forms are increasing day by day in the market and gaining interest due to greater patient acceptability, multiple action and quicker relief. Thus the needs for reliable methods for their analysis are highly demanded.

The aim of this study was to develop a simple yet precise and accurate spectrophotometric method for the simultaneous determination of amlodipine and atorvastatin in tablets dosage forms.

Beer- lambart low was obeyed in the range of 2-10µg/ml and 4.5-23µg/ml for amlodipine and atorvastatin respectively at the selected wavelengths 244nm and 365nm. The proposed method was based on calculating absorption factor, which was calculated by measuring the absorbance of the two analytes in two different wavelengths, in the first wavelength both analyts have absorbance while in the other only one analyte has absorbance, the concentrations of the two drugs in the mixture was calculated using this factor. The accuracy of this method was confirmed by analyzing laboratory synthetic mixtures mean recovery was 99.78±0.65 for amlodipine and 101.31±1.47 for atorvastatin. The precision of the method was evaluated by analyzing commercial tablets on two different days, the repeatability RSD% was found to be 0.432% and 0.336% for amlodipine and atorvastatin respectively, the intermediate precision RSD% was 0.947% for amlodipine and 0.422% for atorvastatin.

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List of abbreviations

AML	Amlodipine
AVS	Atorvastatin
HPLC	High Performance Liquid Chromatography
LOD	Limit Of Detection
LOQ	Limit Of Quantification
RSD	Relative Standard Deviation
SD	Standard Deviation
UV	Ultra Violet
Λ	Lamda

CHAPTER ONE

INTRODUCTION

1.1 Introduction:

Chemical compound absorbs, transmits, or reflects light in a certain range of wavelength, the measurement of how much a chemical substance absorbs or transmits the light is defined as spectrophotometry. It measures how much a chemical substance absorbs light by measuring the intensity of light as a beam of light passes through sample solution. The use of spectrophotometry in quantitative analysis is increasing and gaining importance due to simplicity in operating the instrument, fast, precise and accurate results obtained [1].

Ultraviolet-visible spectrophotometry is a method of analysis that depends on the relation between the analyte concentration and the amount of light absorbed. The amount of this light depends on the electron excitation from low energy level to a higher one within the molecule after passing a beam of light in the range of 200nm-700nm. The leaner relation between the concentration and the absorption is expressed mathematically by Beer-lambert low

$$A=abc$$

Where: A is absorbance and its unit is dimensionless, c concentration has units of moles per liter (M) or gram per 100ml, b is path length in centimeters (cm), a is the absorptivity expressed on the units of concentration used [1].

Ultra- visible spectrophotometry has been used in pharmaceutical analsys; qualitatively if any recorded data is available, quantitatively to ascertain the amount of the drug molecule present in the drug formulation and in impurities detection in these formulations [1].

Quantitative assay of the drug formulation is carried out by preparing a solution in transparent solvent and measure the absorbance at a specific wavelength; this wavelength is selected where there is a maximum absorption (λ_{max}) and the concentration is adjusted to give an absorbance of 0.4-0.9 around which accuracy and precision of the measurements are optimal [1].

The assay of single analtye sample is carried out directly by measuring the absorbance of the sample and then calculating its concentration by one of three principle procedures. They are:

- Standard absorptivity value in which A (1%,1cm) or ε standard values are used and compensated in beer-lamberts formula, this procedure is used when it is expensive or difficult to obtain a pure reference sample.

- Calibration graph in which the absorbances of series of standard solutions of the reference sample at different concentrations encompassing the sample concentration are measured and a calibration graph is constructed. The concentration of the analyte in the sample solution is read from the graph as the concentration corresponding to the absorbance of the solution.
- Single point standardization it involve the measurement of the absorbance of a sample solution and of a standard solution of the reference sample. The concentration of the sample is calculated from the proportional relationship that exists between the absorbance and the concentration.

  $C_{test} = (A_{test} * C_{st})/A_{st}$

  Where: C□$_e$□□ and C□□ are the concentration s of the sample and standard solution respectively and A□$_e$□□ and A□□ are the absorbance of the sample and the standard solution respectively [1].

In the present day many drugs are formulated in a form of two or three active ingredients to achieve a greater efficacy and safety, more patient compliance, fewer side effects and lower cost drug form. Therefore, the need for a simple, cheap, effective and less time consuming way of analysis has become an important matter and a topic of interest for most chemical analysts [2].

The assay of a sample containing more than one analyte presents a challenge for the analyst as most of these analytes absorbs light in the same spectral region which will result in an overlapping of the spectrum making it difficult to use one of the previous procedures in the determination of their concentrations. Traditionally methods of extraction were used to separate the analytes from each other but this was difficult and troublesome as these methods consume large volume of solvent along with the risk of sample loss, contamination or incomplete separation, time consuming and expensive methods [2].

UV spectrophotometric techniques for multi-component analysis have been developing in the past years along with other different analytical techniques that can be used for the analysis of multi-component drugs to overcome the difficulties and errors arising from the traditional methods of separation of the drug formula [2].

When there is no extensive spectral overlapping between the analytes, spectrophotometric methods based on simple mathematical manipulation of the spectral data were used:

- Simultaneous equation method; in this method a set of simultaneous linear equations is solved mathematically to determine the concentration of several components present in the same mixture.
- Absorption ratio method; it depends on the property that for a substance, which obeys beer's low at all wavelength, the ratio of absorbance at any two wavelengths is a constant value independent of the concentration or path length. This ratio is referred to as Q value.
- Difference spectrophotometry; the essential feature of this method is that the measured value is the absorbance difference (ΔA) between two equimolar solutions of the analyte in different chemical forms which exhibit different spectral characteristics.
- Derivative spectrophotometry; it involves the conversion of the normal spectrum (zero order spectrum) to its first, second or higher derivative spectra by differentiating absorbance of the sample with respect to wavelength [3].

Amlodipine besylate (AML), is a white or almost white powder which is slightly soluble in water, freely soluble in methanol [4]. Chemically it is 3-Ethyl-5-methyl (4RS0-2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-6-methyl-1,4–dihydropyridine-3,5- dicarboxylate benzenesulphonate (Figure 1). It has been used in the management of hypertension as it blocks calcium ions transmembrane influx into vascular smooth muscles and cardiac smooth muscles [5].

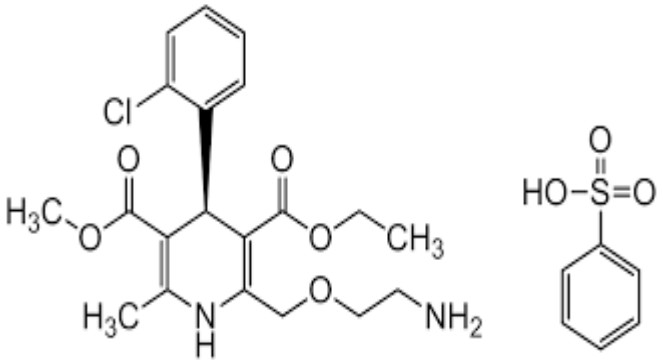

Figure 1: amlodipine besylate chemical structure (mwt: 567.05g/mol).

Atorvastatin calcium (AVS), is a white or almost white powder which is very slightly soluble in water [4]. Chemically it 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 (Figure 2). 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 [5].

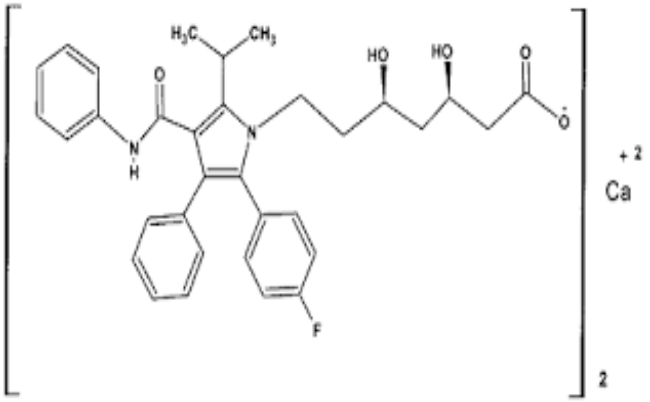

Figure 2: chemical structure of atorvastatin calcium (mwt: 1155.363g/mol).

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 [6].

1.2 Justification:

The individual spectrum of AML and AVS (Figure 3) shows considerable overlapping in the range of 230nm to 290nm, which makes it difficult to apply the classical spectrophtometric techniques for their determination in combined dosage forms.

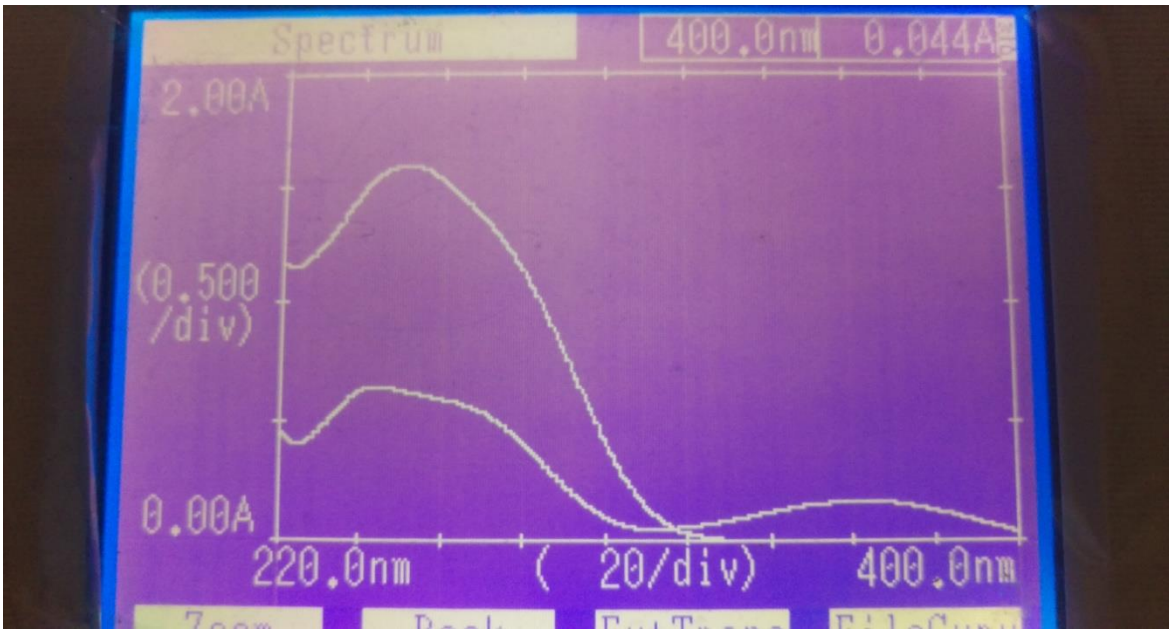

Figure 3: overlay spectrum of amlodipine and atorvastatin

1.3 Objective:

The objectives of this research were:

1. To develop a new spectrophotometric method based on the use of absorption factor to overcome the problem of overlapping spectra
2. To apply the developed method for the estimation of AML and AVS in their commercially available combined tables.

1.4 Literature review:

The assay of amlodipine and atorvastatin combination in drug formulations is not official in any pharmacopeia; however, several methods for their analysis depending on different analytical techniques have been cited.

Multi-component mode of analysis in UV- spectrophotometry, has been used for the simultaneous determination of the two drugs in tablet dosage forms [7, 8].

Principal component regression (PCR) and partial least squares (PLS) chemometric spectrophotometric techniques were developed for the simultaneous determination of AML and AVS, a series of mixtures containing different concentration of the two drugs were prepared and the absorbance value for these mixture at different wavelengths were recorded and the PCR and PLS algorithms were applied for the analysis of the data obtained [9].

Another method based on PCR and PLS was also developed where data was utilized to enhance results and to remove the interference from the sample matrix, along with an HPLC method; mobile phase consisted of methanol and 0.01M sodium dihydrogen phosphate buffer (75:25%, v/v), C_{18} as column and a flow rate at 1.2ml/min [10].

Simultaneous equation method and Q-value analysis at 238.8 nm as isoabsorptive point were used for the determination of the two drugs in tablets [11].

Another Q-value method using 293nm as the isoabsorbative point and 247nm as λ_{max} of AVS was also mentioned for the determination of AML and AVS [12].

First derivative of the ratios spectra by measurement of amplitudes at 228 and 245nm for AML using 25µg/ml of AVS as a divisor and at 284 and 295nm for AVS using 80µg/ml of AML as a divisor, multivariate spectrophhotometric calibration based on (PLS) regression analysis and (HPLC) on C_{18} reversed phase column an acetonitrile: 0.05M KH_2PO_4 (60:40v/v) as mobile phase adjusted by phosphoric acid to pH 3.5 at flow rate of 1ml/min were cited for the simultaneous determination of the two drugs [13].

H-point standard addition method (HPSAM) was used for simultaneous determination of AML and AVS, 241-252.4nm and 278-305.6nm were chosen for the estimation of AML and AVS respectively. The method involves the addition of analyte of interest in the binary mixture, measuring the absorbance at the two wavelengths and a calibration curve is used to calculate the concentration of the main analyte and the interfernt one [14].

Ratio difference, bivariate and absorbance ratio spectrophotometric methods were also used for the determination of the two analytes in tablets [15].

Spectrofluorimetric method and HPLC method with florometric detection were developed for simultaneous determination of both drugs in tablets. In the spectrofluorimetric method, native fluorescence of AML and AVS were measured in methanol at 442 and 369 nm upon excitation at 361 and 274 nm, respectively. In the HPLC method, separation of AML and AVS was achieved within 8 minutes on a C18 column using acetonitrile: phosphate buffer (0.015 M, pH 3) (45:55, v/v) as the mobile phase. Fluorescence detection was carried out using excitation wavelengths 361 and 274 nm and emission wavelengths 442 and 378 nm for AML and AVS, respectively [16].

1.5 Theoretical background:

Absorption factor spectrophotometric method [17], 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 λ_{max} of Y (λ_1), and has no absorption at another wavelength (λ_2) that Y show absorbance at; the absorbance of the mixture at (λ_2) equals the absorbance of Y.

Absorbance of Y at λ_1 = $(\frac{absY_{\lambda_1}}{absY_{\lambda_2}}) \times abs_{\lambda_2}(x+y)$

Absorbance of X at $\lambda_1 = abs_{\lambda_1}(X+Y) - (\frac{absY_{\lambda_1}}{absY_{\lambda_2}}) \times abs_{\lambda_2}(x+y)$

Where: abs: absorbance value, $(\frac{absY_{\lambda_1}}{absY_{\lambda_2}})$: is the absorption factor and it is constant for pure Y.

The concentration of Y or X separately is calculated from their corresponding regression obtained by plotting the absorbance of either X or Y at λ_1 against their corresponding concentration.

CHAPTER TWO

MATERIALS AND METHODS

2. Materials and methods:

2.1 Chemicals:

- Amlodipine besylate and atorvastatin calcium working standards were supplied by Amipaharma Pharmaceuticals Industry – Sudan.
- Methanol analytical grade. (Carlo Erba, Italy)
- Distilled water was provided by the university laboratory.
- Lorvast plus® tablets (B.N. C401) manufactured by Tabuk Pharmaceuticals, Sudan, it is labeled to contain 20 mg of Atorvastatin as calcium salt and 5 mg of Amlodipine as besylate salt and were purchased from the local market.

2.2 Reagents:

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

2.3 Instruments:

- A single beam UV-Visible spectrophotometer UV 1800 (SHMADZU, Japan).

2.4 Preparation of Amlodipine standard stock solution:

15 mg of amlodipine working standard were accurately weighed and transferred into a 100 ml volumetric flask the volume was completed to the mark using 50% aqueous methanol to have a final concentration of 150µg/ml.

2.5 Preparation of Atorvastatin standard stock solution:

25 mg of atorvastatin working standard were accurately weighed and transferred to a 100 ml volumetric flask the volume was completed to the mark with 50% aqueous methanol to have a final concentration of 250µg/ml.

2.6 Selection of analytical wavelengths:

5 ml aliquot from standard stock solution of amlodipine and atorvastatin were transferred to two separate 50 ml volumetric flask, and made up to the mark using the diluent. The individual spectra of the two solutions were obtained over the wavelength range of 200-400nm.

2.7 Linearity and calibration:

Serial dilutions were prepared from each standard stock solution of the drugs by diluting 1, 2,3,4,5 ml in five different 50 ml volumetric flasks with the diluents, to give amlodipine in the range of 2-10µg/ml and atorvastatin in the range of 4.5-23µg/ml. The absorbance of these solutions was measured at the selected wavelengths and a calibration curves were obtained.

2.8 Preparation of synthetic mixtures:

Nine laboratory synthetic mixtures containing different concentrations of amlodipine and atorvastatin were prepared according to [18] by mixing different volumes from the two stock solutions in nine 50 ml volumetric flask and making the volume to the mark with the diluent. The scheme describing the method of preparation of these mixtures and the corresponding concentrations of the two drugs in the mixtures are shown in Tables 1and 2 below.

Table 1: Synthetic mixtures preparation scheme

mixture	AML	AVS
1	-1	-1
2	0	0
3	1	1
4	-1	0
5	0	-1
6	1	-1
7	-1	1
8	0	1
9	1	0

Table 2: concentrations of the analytes in the synthetic mixtures

Mixtures	AML conc in µg/ml	AVS conc in µg/ml
1	4.384	8.333
2	6.576	12.499
3	8.768	16.665
4	4.384	12.499
5	6.576	8.333
6	8.768	8.333
7	4.384	16.665
8	6.576	16.665
9	8.768	12.499

2.9 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 minutes and filtered using 0.45 micron nylon syringe filter, 5 ml of the filtrate were diluted to 50 ml using the diluent.

CHAPTER THREE

RESULTS AND DISCUSSION

3. Results

3.1 Selection of analytical wavelength:

The individual spectrum of amlodipine and atorvastatin obtained over the wavelength range of 200-400nm using a 15µg/ml solution of amlodipine and 25µg/ml solution of atorvastatin, showed that amlodipine is having absorbance maxima at 244nm and 365nm, while atorvastatin is having one absorbance maxima at 244nm and no absorbance at 365nm, hence these two wavelengths (244nm and 365nm) were selected for the application of this method.

3.2 Linearity over the selected wavelengths:

The absorbances of amlodipine at 244nm and 365 nm and of atorvastatin at 244nm were plotted against their corresponding concentrations in order to obtain the calibration curves, linear regression analysis of the plotted data indicate that there is a good correlation between absorbance and concentration for both analytes (r^2 >0.999). The regression analysis data of the two analytes and the corresponding calibration plots are shown in Tables 3- 5 and Figures 3-5.

The limit of quantification and limit of detection were evaluated from the regression data using the following equation:

$LOQ=10*\sigma/S$

$LOD=3.3*\sigma/S$

LOQ= limit of quantification, LOD= limit of detection, σ= the standard deviation of the response, S= the slop of the calibration curve.

Absorption factor was calculated as an average from the absorbance data of amlodipine standard solution at the two wavelengths it was found to be 2.7042. This factor is used in the subsequent calculations involving the corresponding absorbance values of the two analytes at the analytical wavelength 244nm.

Table 3: AML standard solution linearity data at 244nm

Solution No	AML conc in µg/ml	Abs at 244nm
1	2.19	0.099
2	4.38	0.2
3	6.57	0.299
4	8.76	0.403
5	10.96	0.498
Slope		0.0456
Intercept		0.0005
Correlation coefficient		0.9999
Residual sum of squares		0.0000147
LOQ		0.509µg/ml
LOD		0.168µg/ml

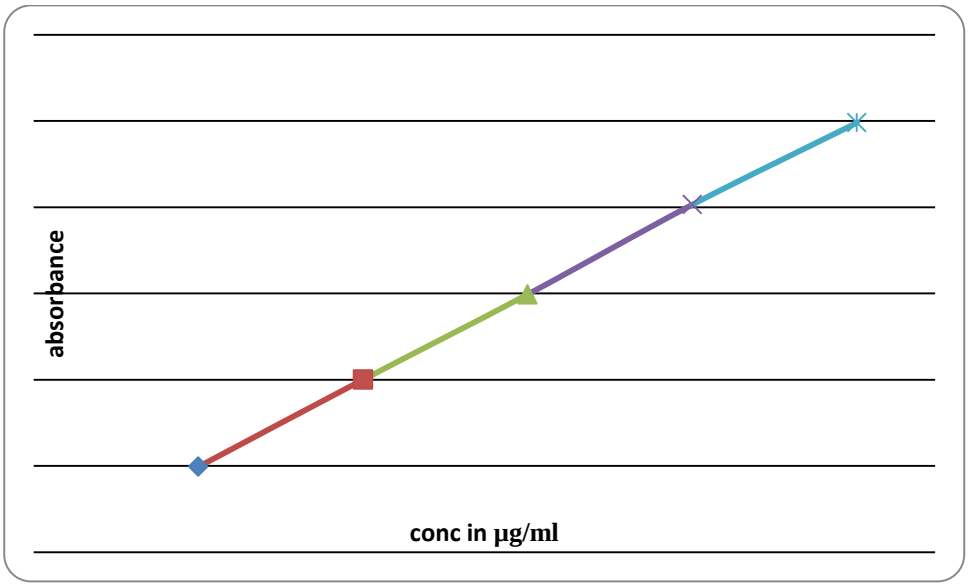

Figure 4: linearity plot of AML at 244nm

Table 4: AML standard solution linearity data at 365nm

Solution No	AML conc in µg/ml	Abs at 244nm
1	2.19	0.039
2	4.38	0.074
3	6.57	0.107
4	8.76	0.145
5	10.96	0.184
Slope		0.0164
Intercept		0.0015
Correlation coefficient		0.9994
Residual sum of squares		0.0000147
LOQ		1.45µg/ml
LOD		0.467µg/ml

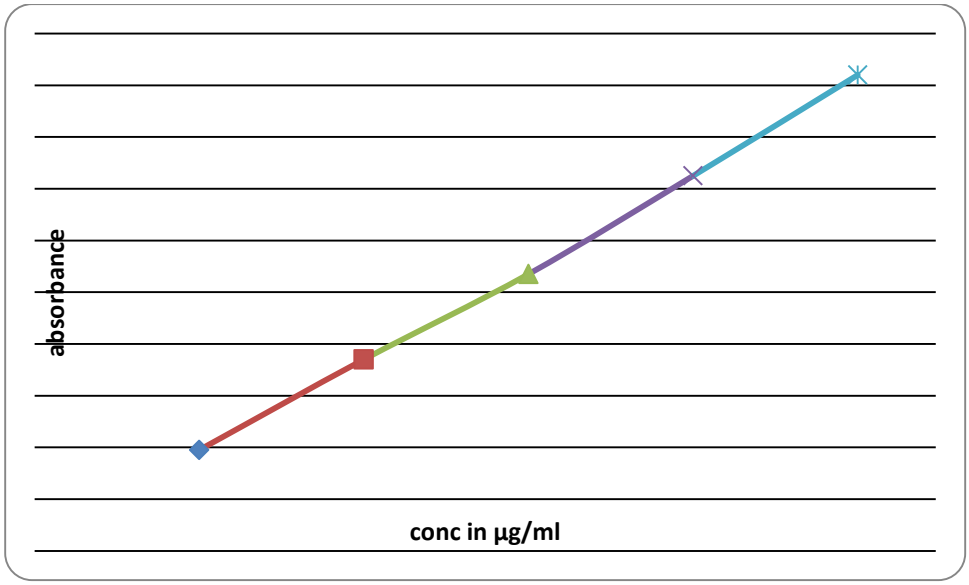

Figure 5: linearity plot of AML at 365nm

Table 5: AVS standard solution linearity data at 244nm

Solution No	AVS conc in µg/ml	Abs at 244nm
1	4.67	0.189
2	9.34	0.78
3	14.02	0.569
4	18.69	0.749
5	23.37	0.937
Slope		0.0399
Intercept		0.0043
Correlation coefficient		0.9999
Residual sum of squares		0.0000303
LOQ		0.8353µg/ml
LOD		0.275µg/ml

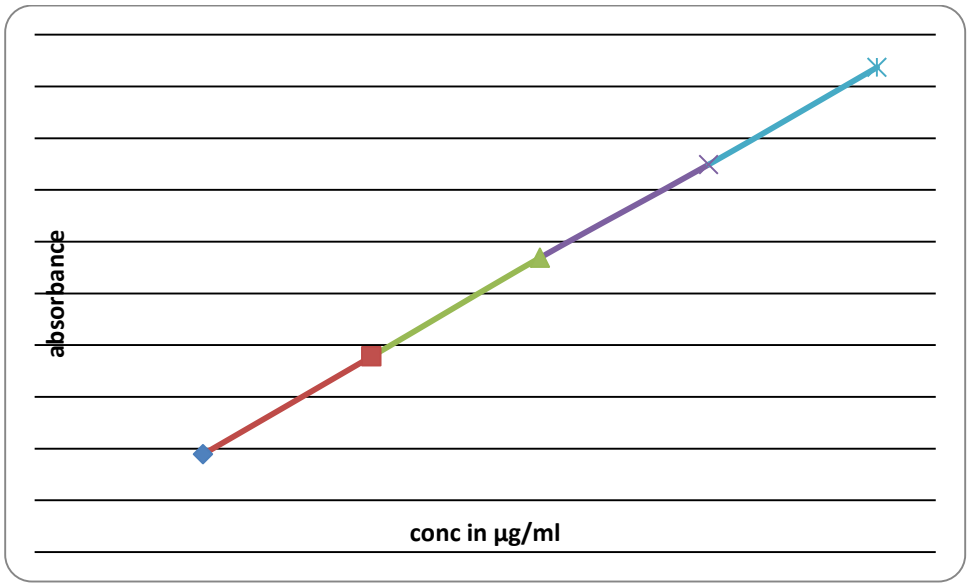

Figure 5: linearity plot of AVS at 244nm

3.3 Accuracy:

This method's accuracy was tested by analyzing the nine laboratory prepared synthetic mixtures of amlodipine and atorvastatin. Using the corrected absorbance at 244nm obtained using the absorption factor of 2.7042, the results of the determination of the two analytes showed good agreement between the theoretical and actual concentrations of the two analytes. Amlodipine average recovery from the synthetic mixtures was (99.78±0.65), while the atorvastatin average recovery was (101.31±1.47). The small relative standard deviation (< 2%) support the accuracy of the method [19].

The absorbance data of the synthetic mixtures at the selected wavelengths, the absorbance of each at 244nm after correction using the absorption factor and the summery of accuracy results are shown in tables 6, 7 and 8 respectively.

Table 6: Absorbance data of the mixtures at the selected wavelengths

Mixture	Abs at 244	Abs at 365
1	0.532	0.074
2	0.799	0.111
3	1.047	0.148
4	0.703	0.075
5	0.627	0.112
6	0.723	0.147
7	0.846	0.074
8	0.965	0.111
9	0.898	0.147

Table 7: The calculated absorbance values of AML and AVS in the mixtures at 244nm

Mixture	Abs of AML*	Abs of AVS*
1	0.201	0.331
2	0.301	0.498
3	0.401	0.646
4	0.203	0.500
5	0.304	0.323
6	0.398	0.325
7	0.201	0.645
8	0.301	0.664
9	0.398	0.500

*calculated using the absorption factor

Table 8: The accuracy results of the synthetic mixtures

Mixture	Amlodipine (µg/ml)		% content	atorvastatin(µg/ml)		% content
	Theoretical	Actual		Theoretical	Actual	
1	4.384	4.37	99.68084	8.333	8.52	102.2524
2	6.576	6.555	99.68084	12.499	12.806	102.4581
3	8.768	8.74	99.68084	16.665	16.604	99.63011
4	4.384	4.429	101.0279	12.499	12.847	102.7807
5	6.576	6.614	100.5789	8.333	8.315	99.78771
6	8.768	8.681	99.00733	8.333	8.344	100.1399
7	4.384	4.37	99.68084	16.665	16.592	99.56191
8	6.576	6.555	99.68084	16.665	17.074	102.4497
9	8.768	8.681	99.00733	12.499	12.843	102.7525
Average			99.78063			101.3126
SD			0.655612			1.471112
RSD%			0.657053			1.452053

3.4 Precision:

The repeatability of the method was evaluated by calculating the % RSD of sample determinations carried out in the same day and was found to be 0.432% and 0.336% for amlodipine and atorvastatin respectively. The small relative standard deviation (<1%) support the precision of the method [19] as shown in Tables 9-11.

Table 9: Samples weight taken and corresponding amount of analyte

Sample	Weight taken(gm)	Active (mg)	
		AML	AVS
1	0.2466	4.99	19.99
2	0.2466	4.99	1 9.99
3	0.2466	4.99	19.99
4	0.2467	5	20
5	0.2467	5	20
6	0.2466	4.99	19.99

Table 10: The absorbance data at the selected wavelengths of the sample and of the individual AML and AVS

Sample	Sample		Individual analytes	
	Abs at 244nm	Abs at 365nm	Abs of AML* at 244nm	Abs of AVS* at 244nm
1	1.019	0.083	0.224	0.795
2	1.019	0.083	0.224	0.795
3	1.018	0.083	0.224	0.794
4	1.019	0.084	0.227	0.792
5	1.019	0.083	0.224	0.795
6	1.018	0.083	0.224	0.794

*calculated using the absorption factor

Table 11: The assay results of the samples

Sample	Amlodipine (µg/ml)		% content	atorvastatin(µg/ml)		% content
	Theoretical	Actual		Theoretical	Actual	
1	4.99	4.915054	98.49807	19.99	19.89311	99.96536
2	4.99	4.915054	98.49807	1 9.99	19.89311	99.96536
3	4.99	4.915054	98.49807	19.99	19.86807	99.83955
4	5	4.974271	99.48542	20	19.8254	99.127
5	5	4.915054	98.30107	20	19.89311	99.46554
6	4.99	4.915054	98.49807	19.99	19.86807	99.83955
Average			98.62979			99.70039
SD			0.426513			0.335584
RSD%			0.432438			0.336593

The intermediate precision was studied by assaying another set of samples in different day following the same method. The %RSD was 0.947% for amlodipine and 0.422% for atorvastatin as shown in Tables 12-14.

The average %RSD for the twelve determinations at the two different days was 0.689% and 0.379% for amlodipine and atorvastatin respectively which are less than 3% as specified by ICH [19].

Table 12: Samples weight taken and corresponding amount of analyte

Sample	Weight taken(gm)	Active (mg)	
		AML	AVS
1	0.2475	5	20
2	0.2472	4.9	19.9
3	0.2476	5	20
4	0.2473	4.99	19.9
5	0.2472	4.99	19.9
6	0.2476	5	20

Table 13: The absorbance data at the selected wavelengths of the sample and of the individual AML and AVS

Sample	Abs at 244nm	Abs at 365nm	Abs of AML* at 244nm	Abs of AVS* at 244nm
1	1.022	0.084	0.227	0.795
2	1.023	0.083	0.224	0.799
3	1.022	0.083	0.224	0.798
4	1.024	0.084	0.227	0.797
5	1.022	0.085	0.230	0.792
6	1.024	0.085	0.230	0.794

*calculated using the absorption factor

Table 14: The assay results of the samples

Sample	Amlodipine (µg/ml)		% content	atorvastatin(µg/ml)		% content
	Theoretical	Actual		Theoretical	Actual	
1	5	4.974	99.48542	20	19.901	99.50256
2	4.9	4.915	100.3072	19.9	19.993	100.4686
3	5	4.915	98.30107	20	19.968	99.84109
4	4.99	4.974	99.68479	19.9	19.951	100.2542
5	4.99	5.033	100.8715	19.9	19.833	99.66233
6	5	5.033	100.6698	20	19.883	99.41439
Average			99.88663			99.8572
SD			0.94614			0.422263
RSD%			0.947214			0.422867

3.5 Analysis of commercial sample:

The method was applied to the analysis of six samples taken from commercial tablets in replicates. The results obtained were in good agreement with the labeled concentration; the recovery average of amlodipine was 99.25% with an average relative standard deviation of 0.689% and the average recovery of atorvastatin was 99.77% with an average relative standard deviation of 0.379%. The analysis data of the sample were displayed in table 10-12.

3.6 Conclusion:

The absorption factor method was found to be accurate, precise, simple and sensitive, hence can be used for the routine analysis for the simultaneous estimation of amlodipine besylate and atorvastatin calcium in their pharmaceutical dosage forms and in processes control.

CHAPTER FOUR

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