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Research Article

Quantitative Determination of Amlodipine and Valsartan in Pharmaceutical Dosage Form

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ABSTRACT

Two simple spectrophotometric methods had been developed for simultaneous determination of amlodipine (AMD) and valsartan (VAL) in their tablet dosage form. These methods include absorption correction method and dual wavelength method. The first method was based on the measurement of absorbance at 360 nm for AMD and 257.1 nm for VAL, while the second depended on measuring the absorbance difference (ΔA) in zero order spectra between (226.5-250 nm) for determination of VAL while AMD was determined directly by measuring absorbance at 360 nm. Both methods were validated according to International Council for Harmonisation (ICH) guidelines. The linearity range was found to be 8–25 µg/mL for determination of AMD and 6–35 µg/mL for determination of VAL. The methods were successfully applied for the simultaneous determination of AMD and VAL in the commercially available Exforge® tablets. The average values of percent recovery ± standard deviation was found to be consistent with the label claim of the dosage form. The results were also compared to a reported method using t-test and F-test at confidence level of 95%, no significant differences were observed. The presented methods permit simple, rapid, and direct determination of AMD and VAL in commercially available combined dosage form using zero-order UV spectra without previous separation and were suitable for routine analysis.

Keywords: Absorption correction method, Amlodipine, Dual wavelength method, UV spectrophotometric method, Valsartan.

1. INTRODUCTION

Hypertension is a widely spread chronic disease which is directly responsible for 51% of all stroke deaths and 45% of all coronary heart diseases worldwide.[1] The goal of antihypertensive therapy is to abolish the risks associated with blood pressure elevation without adversely affecting quality of life. Drug selection is based on efficacy in lowering blood pressure and in reducing cardiovascular end points including stroke, myocardial infarction, and heart failure.[2] Combination drug products occupy a time honored and important role in therapeutics when rationally formulated. Fixed dose combination drugs may produce greater convenience, lower cost, and sometimes greater efficacy and safety. Antihypertensives' combination products are intended to be formulated to achieve certain benefits as; treatment of hypertension with different mechanisms or treatment of multiple diseases associated with hypertension.[3]

Amlodipine Besylate (AMD) **(Figure 1A)** is a calcium channel blocker. It is a third generation dihydropyridine calcium antagonist. Chemically it is 3-ethyl 5 –methyl 2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-6-methyl-1, 4-dihydropyridine-3.5-dicarboxylate]. Several fixed dose combination (FDC) combinations of AMD and other drugs are used to enhance their activity. AMD is a white crystalline powder. Its solubility in water and propanol is very poor. It is freely soluble in methanol and sparingly soluble in ethanol. Its

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pKa is 8.6. The melting range for AMD is between 195 – 204^{O}C.[2,4,5]

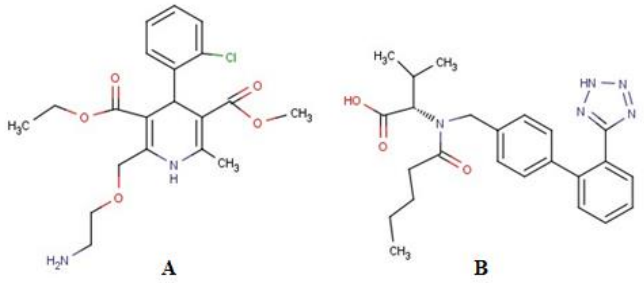

Figure 1: Chemical structure of AMD (A) and VAL (B)

Valsartan (VAL) **(Figure 1B)** is a potent, highly selective, and orally active antihypertensive drug. It is one of the family of angiotensin II type I receptor antagonists which have been used in the treatment of hypertension, heart failure and myocardial infarction.[6] Chemically it is (1S,3R,7S,8S,8aR)-8-{2-[(2R,4R)-4-hydroxy-6-oxooxan-2-yl]}-3,7-dimethyl1,2,3,7,8,8 ahexahydronaphthalen-1-yl 2,2 –dimethyl butanoate.[4] VAL is a white powder. It is very soluble in methanol and ethanol and practically insoluble in water. Its melting range is 105-110 °C. VAL is a tetrazole derivative that contains two weakly acidic function groups (tetrazole hydrogen and carboxylic acid) with pK_a value of 4.7 and 3.9. These groups make VAL stable in the neutral pH range.[6]

The AMD/VAL FDC achieved significantly greater reductions in mean sitting diastolic and systolic blood pressure than AMD or VAL monotherapy. Moreover, the incidence of peripheral oedema was significantly lower in patients receiving the FDC than in those treated with AMD monotherapy.[7]

The review of literature revealed several methods reported for determination of AMD alone or in combination. These methods include UV spectrophotometry,[8-12] colorimetry,[13-15] HPLC,[16,17] and HPTLC.[18] For VAL, UV spectrophotometry,[19-22] HPLC,[19,23] and HPTLC[24] methods were reported. Published methods for simultaneous determination of AMD and VAL include UV spectrophotometric,[25-36] spectrofluorimetric methods[37-39], HPLC,[25,30,31,40-47] TLC[44,48] and capillary electrophoresis.[49,50]

The present research work is concerned with the development of two simple, accurate and sensitive spectrophotometric methods: absorption correction method (ACM) and dual wavelength method (DWM) for simultaneous determination of AMD and VAL with no need for prior separation. The presented methods depend on extracting quantitative data from the fundamental zero-order UV spectra, without need for calculating mathematically manipulated derivative, ratio and /or subtraction spectra. They are based on measuring absorbance at two wavelengths and then concentrations can be calculated after solving simple equations. The developed spectrophotometric methods are simpler than the reported chromatographic and electrophoretic methods as there is no need for expensive solvent, sophisticated instruments, or complicated procedures.

2. MATERIALS AND METHODS

2.1. Apparatus and software

A Shimadzu UV-1800 UV/Vis double beam (Kyoto-Japan) spectrophotometer with 1cm quartz cells was used. It was connected to a personal computer loaded with UV probe ver.2.33 software. Zero order spectra were recorded in the wavelength range 200-450 nm at 0.1 nm sampling intervals. Microsoft excel was used for different calculations.

2.2. Materials

AMD (99.00%) and VAL (96.00%) were kindly donated by Sigma for Pharmaceutical Industries (Quesna-Egypt). Methanol analytical grade (Merck, Germany) was used. The commercially available Exforge® (10-160) tablets were purchased from a local market (batch number: Y0114). Each tablet is labeled to contain 10 mg of AMD and 160 mg of VAL.

2.3. Standard solutions

2.3.1. Stock standard solution

Stock standard solutions of AMD and VAL containing (1mg/mL) were prepared by transferring accurately weighed 10 mg of each into two separate 10-mL volumetric flasks, dissolved in methanol and the volumes were completed with the same solvent.

2.3.2. Working standard solutions

Different aliquots were taken from stock standard solutions and diluted with methanol to obtain working standard solutions containing 100 µg/mL of AMD and 40 µg/mL of VAL. AMD standard solutions were protected from light due to its photosensitivity by covering them by aluminum foil. The standard solutions were stable for 10 days when kept in the refrigerator based on that the calculated % recovery is not less than 98% during storage period.

2.4. Construction of calibration curves

A set of laboratory-prepared standard solutions of AMD and VAL were prepared by transferring different aliquots of working standard solution into 10-mL volumetric flask and diluting to volume with methanol to obtain solutions containing 8-25 µg/mL of AMD and 6-35 µg/mL of VAL.

Zero order absorption spectra were recorded against methanol as a blank and stored in the computer. AMD can be

determined directly at its λ_{max} (360 nm) where VAL does not show any interference at this wavelength. VAL can be determined by two methods:

2.4.1. Absorption correction method (ACM)

Calibration curve for VAL was constructed by plotting the absorbance at 257.1 nm versus the corresponding concentrations and regression equation was computed. For simultaneous determination of AMD and VAL in a laboratory-prepared binary mixture or tablet assay solution, absorbance at 360 nm is subtracted from that at 257.1 nm before calculating the concentration of VAL from its regression equation.

2.4.2. Dual wavelength method (DWM)

VAL calibration curve was obtained by plotting the absorbance difference (ΔA) between 226.5 and 250 nm versus the corresponding concentrations and regression equation was computed.

2.5. Analysis of both drugs in pharmaceutical dosage form

Ten Exforge® tablets were accurately weighed and finely powdered in a mortar. An amount equivalent to one tablet content (10 mg of AMD and 160 mg of VAL) was transferred to 100-mL volumetric flask and diluted with methanol. The solution was kept sonicated for 20 minutes, completed to the volume with methanol and then, filtered through Whatman filter paper (No. 41). The first 10 mL aliquot of filtrate was discarded. An aliquot equivalent to 10 mL of the previous tablet stock solution was transferred to a 100-mL volumetric flask and completed to volume with methanol. Then, 1 mL of this solution was transferred to a 10 mL volumetric flask, spiked with an 0.9 mL of 100 µg/mL AMD working standard solution, and the volume was completed to 10 mL with methanol. The prepared diluted tablet assay solution is then claimed to contain 10 µg/mL AMD and 16 µg/mL VAL. The solution was analyzed in triplicate by the developed methods. The claimed concentration of AMD in tablet was calculated after subtraction of the added concentration (9µg/mL previously analyzed by the method developed by Shah et al[12] and found to be equivalent to 9.002 µg/mL).

3. RESULTS AND DISCUSSION

UV spectrophotometric methods offer simple, rapid, cost effective and time saving alternatives to HPLC methods. However, direct determination of UV active drugs in binary mixtures is only possible if both drugs are completely resolved at λ_{max}. Mathematically assisted spectrophotometric techniques are used to face challenges concerning the simultaneous analysis of drugs with overlapped UV spectra. As shown in Figure (2), AMD can be determined directly at its λ_{max} (360 nm). VAL cannot be determined directly from UV spectra due to severe overlap with AMD at its wavelength of maximum absorption. Two spectrophotometric methods were tried, ACM and DWM for solving this spectral overlap.

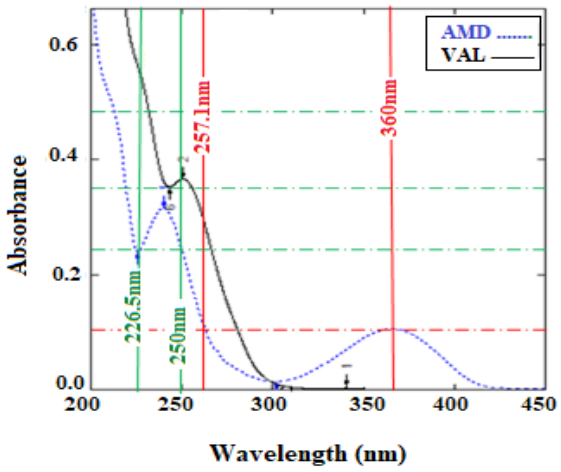

Figure 2: Zero-order UV absorption spectra of AMD (10 µg/mL) and VAL (16 µg/mL) in methanol.

3.1. Theory of the developed UV-spectrophotometric methods

3.1.1. ACM

This method describes the analysis of a binary mixture where the two components "X" and "Y" have overlapped spectra. Y shows interference at λ_{max} of X (λ_1), while X shows no interference with Y at another wavelength (λ_2). Two wavelengths from spectra, λ_1 and λ_2 were selected where Y shows equal absorbance at both wavelengths. At λ_2, estimation of Y was done easily where no interference of X occurs, Here the absorbance of Y at λ_1 equals that at λ_2. Concentration of X can be easily calculated at λ_1 after subtraction of Y contribution at this wavelength. The absorbance for X is called the corrected absorbance.[51]

For the simultaneous determination of AMD and VAL using ACM **(Figure 2)**, two wavelengths were selected λ_1 257.1 nm and λ_2 360 nm, at which AMD exhibits the same absorbance. AMD could be determined directly without any interference from VAL at 360 nm (equations 1-4). Quantitation of VAL in a mixture at 257.1 nm can be done by subtracting the absorbance of AMD at 360 nm from the absorbance of mixture at 257.1 nm (equations 5 and 6).

$$A_{mix.\ at\ 360nm} = A_{AMD\ at\ 360nm} \quad (1)$$

$$A_{mix.\ at\ 257.1nm} = A_{AMD\ at\ 360nm} + A_{VAL\ at\ 257.1nm} \quad (2)$$

$$A_{mix.\ at\ 360nm} = a_{AMD\ at\ 360nm} . b . C_{AMD} \quad (3)$$

$$\mathbf{C_{AMD}} = \frac{\mathbf{A_{mix.at\ 360nm}}}{\mathbf{a_{AMD\ at\ 360nm}} \cdot \mathbf{b}} \quad (4)$$

$$A_{VAL\ at\ 257.1nm} = A_{mix.\ at\ 257.1nm} - A_{AMD\ at\ 360nm} \quad (5)$$

$$\mathbf{C_{VAL}} = \frac{A_{mix.\ at\ 257.1nm} - A_{AMD\ at\ 360nm}}{\mathbf{a_{VAL\ at\ 257.1nm}} \cdot \mathbf{b}} \quad (6)$$

Where **A** is the absorbance, **a** is the absorptivity, **b** is the pass length and **C** is the concentration.

3.1.2. DWM

Dual wavelength spectroscopy offers an efficient method for analyzing a component in presence of an interfering component. In simultaneous analysis of a binary drug mixture, one drug was considered as a component of interest while the other was considered as an interfering component. For elimination of interference, two wavelengths were selected for the drug in a way so that the difference in absorbance is zero for the other. The absorbance difference (ΔA) between two points in the mixture spectra is directly proportional to the concentration of the component of interest independently of the interfering component.[52]

VAL was determined by measuring ΔA between 226.5 nm and 250 nm; at which ΔA for AMD equals zero. AMD was determined directly at 360 nm **(Figure 2)**.

3.2. Method validation

Methods were validated according to ICH guidelines.[53] The following validation parameters were addressed.

3.2.1. Linearity and range

The linearity of the developed methods was assessed by plotting the concentrations of AMD against absorbance measured at 360 nm (A_{360nm}). For VAL, concentration is plotted against absorbance measured at 257.1 nm ($A_{257.1nm}$) in ACM and absorbance difference between 226.5 and 250 nm ($dA_{226.5-250nm}$) in DWM. Regression analysis was carried out using Microsoft Excel. Regression parameters for determination of AMD and VAL by the developed method are shown in Table (1). The high values of correlation coefficient (r) with small values of standard deviations around intercept, S_a, slope, S_b and the residuals, $S_{y/x}$ indicate the acceptable linearity over the ranges of 8-25 µg/mL for AMD and 6-35 µg/mL for VAL.

3.2.2. Limits of detection and quantitation

The LOD and LOQ for AMD and VAL were calculated in Table (1) using standard deviation of intercept (Sa) and the slope of calibration curve (b) as follows:

$$LOD = 3.3Sa / b$$

$$LOQ = 10Sa / b$$

The calculated values for LOD were found to be 0.689 µg/mL for AMD and 0.402 and 0.429 µg/mL for VAL by ACM and DWM respectively which indicates that the methods have sufficient sensitivity for determination of AMD and VAL in bulk, diluted solutions, and pharmaceutical dosage forms.

Table 1: Regression parameters for determination of AMD and VAL using the proposed methods.

Parameter	AMD	VAL	
		ACM	DWM
Linearity range(µg/mL)	8-25	6-35	6-35
λ (nm)	360	257.1	226.5-250
r	0.9995	0.9997	0.9998
a	-0.0017	0.0024	0.0031
b	0.0124	0.0255	0.0221
S_a	0.0026	0.0031	0.0029
S_b (10^{-2})	0.0134	0.0153	0.0141
$S_{y/x}$ (10^{-2})	0.0232	0.0416	0.0115
LOD (µg/mL)	0.689	0.402	0.429
LOQ (µg/mL)	2.089	1.219	1.299

r: correlation coefficient, a: intercept, b: slope, S_a: standard deviation of intercept, S_b: standard deviation of slope, $S_{y/x}$: standard deviation of residuals, LOD: limit of detection (calculated), LOQ: limit of quantitation (calculated).

3.2.3. Accuracy

To assess the accuracy, the developed methods were applied to three binary mixtures containing different concentrations of AMD and VAL (three replicates) covering the linearity range of each and the % recovery was calculated **(Table 2)**. The high value of % recovery with the small value of S.D. proves the methods' accuracy.

3.2.4. Precision

The precision of the methods was studied by testing intra-day and inter-day precision. Three binary mixtures containing different concentrations of AMD and VAL covering the linearity range of each were used. Intra-day precision was evaluated by determination of three replicates of the binary mixtures in the same day. The same procedures were repeated in three consecutive days to study the inter-day precision. As shown in Table (3), the small values of % relative standard deviation (% R.S.D <2.0%) prove the precision of the methods.

Table 2: Evaluation of accuracy for the determination of AMD and VAL by the proposed spectrophotometric methods.

Drug	Concentration added (µg/mL)	Concentration found (µg/mL)			Mean concentration found (µg/mL)	% Recovery	Mean % Recovery ± S.D
AMD	10	9.925	9.889	9.985	9.890	98.90	99.000 ± 0.351
	15	14.856	14.742	14.821	14.806	98.71	
	20	19.961	19.732	19.942	19.878	99.39	
VAL (ACM)	16	16.012	16.038	15.799	15.949	99.69	99.890 ± 0.436
	20	20.011	20.241	19.985	20.079	100.39	
	25	25.027	24.976	24.694	24.899	99.59	
VAL (DWM)	16	15.908	15.896	16.12	15.975	99.84	99.880 ± 0.040
	20	20.001	19.955	19.974	19.977	99.88	
	25	24.995	24.982	24.963	24.98	99.92	

*n=3, S.D, standard deviation; % R.S.D, percentage relative standard deviation.

Table 3: Evaluation of intra-day and inter-day precision for the determination of AMD and VAL by the proposed spectrophotometric methods.

Drug (Method)	Concentration added (µg/mL)	Intra-day			Inter-day		
		Mean concentration found* (µg/mL)	S.D	% R.S.D	Mean concentration found* (µg/mL)	S.D	% R.S.D
AMD (Direct)	10	9.999	0.024	0.240	9.986	0.013	0.130
	15	14.959	0.057	0.381	14.988	0.031	0.207
	20	20.012	0.069	0.345	19.975	0.042	0.210
VAL (ACM)	16	15.962	0.057	0.357	15.944	0.044	0.276
	20	19.966	0.034	0.170	19.927	0.036	0.180
	25	24.929	0.033	0.132	24.975	0.040	0.160
VAL (DWM)	16	15.958	0.0365	0.229	15.979	0.020	0.125
	20	19.932	0.0527	0.265	19.943	0.053	0.266
	25	24.975	0.0205	0.082	25.001	0.027	0.107

*n=3, S.D, standard deviation; % R.S.D, percentage relative standard deviation.

3.2.5. Specificity

According to ICH-Q2R1 guidelines, the method is specific if it is not affected by the presence of expected matrix interferences, e.g., excipients, impurities, and degradants. To assess methods' specificity, the UV spectrum of a binary mixture containing both drugs is compared to that of tablet assay solution containing the same concentration of AMD and VAL **(Figure 3)**. Also, when the developed methods are applied for the determination of both drugs in tablet dosage form, acceptable % recovery ± S.D with small value of % R.S.D prove the methods' specifity **(Table 4)**.

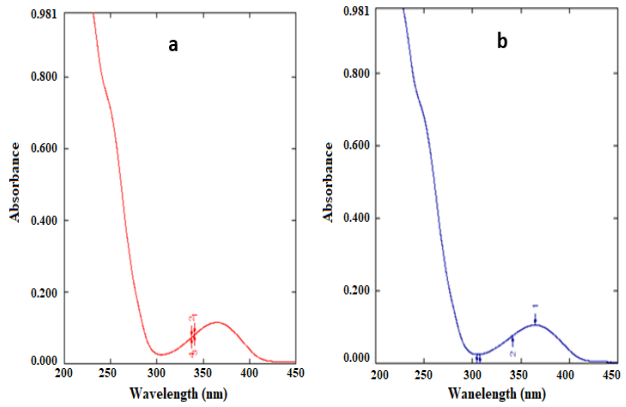

Figure 3: UV spectrum of standard binary mixture (a), and tablet assay solution(b), both containing 10 µg/mL AMD and 16 µg/mL VAL in methanol

Table 4: Determination of AMD and VAL in Exforge® tablets[a] by the developed methods and the reported spectrophotometric method.

Drug (Method)	Developed methods	Reported method[26]	t-test[c]	F-test[c]
	Mean[b] %recovery ± S.D	Mean[b] %recovery ± S.D		
AMD (Direct)	99.700 ± 0.911	99.200 ± 0.511	0.189	3.171
VAL (by ACM)	100.086 ± 0.884	99.410 ± 0.509	0.072	3021
VAL (by DWM)	99.680 ± 0.583		0.278	1.313

[a] Exforge® tablets labeled to contain 10 mg of AMD and 160 mg of VAL.
[b] Mean percentage found of four different sample solutions.
[c] Theoretical values for t-test (0.05) is 2.353 and for F-test (0.05) is 9.276.

3.3. Analysis of pharmaceutical dosage form

The developed methods were used for determination of both drugs in Exforge® tablets claimed to contain 10 mg of AMD and 160 mg of VAL. The problem arises during the analysis of this mixture is that the minor component (AMD) is also the weakly absorbing component. This makes the determination of both drug in the ratio of the dosage form (1AMD:16 VAL) a challenging issue. To facilitate determination of the minor component, its concentration in the tablet assay solution should be increased using sample enrichment technique.[54,55] This is done by adding a fixed amount of a standard AMD (equivalent to 9 µg/mL) to each experiment to increase its concentration to be within the linearity range of the method. Then, after determination, the added amount of standard (which is previously analyzed by the method developed by Shah et al[12]) is subtracted from the taken concentration.

The assay solution of the tablet was analyzed using the developed methods. Accepted values of % recovery ± S.D and low value of % R.S.D indicated that the methods were successfully used to determine both drugs in tablet dosage form. A reported spectrophotometric method[26] was used for determination of both drugs in pharmaceutical tablets. The reported method is based on applying the simultaneous equation method which is based on writing Beer's expression of the mixture at two wavelengths, which are the wavelength of maximum absorption for each drug, and two equations were solved simultaneously for calculating drugs' concentrations. The results of the developed methods were statistically compared to those obtained by the reported method using the student's t-test and F-test. The experimental t- and F- values did not exceed the theoretical values at 95% confidence level, indicating the absence of any significant difference between the developed and comparison methods **(Table 4)**.

4. CONCLUSION

This work describes two simple spectrophotometric methods for simultaneous determination of AMD and VAL. These are absorption correction (ACM) and dual wavelength (DWM) methods. AMD is determined directly from zero-order UV spectrum at its wavelength of maximum absorption. VAL determination is based on measuring absorbance at two wavelengths, either by calculating the corrected absorbance at λ_{max} in ACM or by measuring ΔA at two selected wavelengths where the ΔA for AMD equals zero in DWM. The presented methods depend on extracting quantitative data from the fundamental UV spectra, without need for calculating derivative, ratio and /or subtraction spectra. The achieved sensitivity is comparable to the previously published spectrophotometric methods and is suitable for determination of AMD and VAL in diluted solutions and in pharmaceutical dosage forms.

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