Simultaneous UV Spectrophotometric Determination of Valsartan and Amlodipine in tablet

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ABSTRACT: In the present work a simple, accurate and precise method has been developed and validated for the simultaneous estimation of Valsartan and Amlodipine and in their combined dosage form by UV Spectrophotometric methods. The Method A employs estimation of drugs by simultaneous equation method (SEM) using 250.0 and 238.0 nm i.e. λ_{max} values of VAL and AMD respectively. Method B employs the estimation of drugs by Absorption Correction method (ACM) at 360.0 i.e. λ_{max} values of one drug and 236.0 nm an isobestic wavelength. VAL and AMD individually and in mixture follow Beer's law over the concentration range 5-30 μg/mL at all the selected wavelengths. Additivity study concluded that both the drugs do not interact with each other in solution. The percent recoveries of the drugs were found nearly 100 % representing the accuracy of the both methods. Validation of the proposed methods was carried out for its accuracy, precision, specificity, linearity and range, ruggedness, limit of detection according to ICH guidelines. The proposed methods can be successfully applied in routine work for the determination of valsartan and amlodipine in combined dosage form.
Keywords: Valsartan (VAL), Amlodipine (AMD), Ultraviolet Spectroscopy, Simultaneous Equation Method, Absorbance Correction Method.

INTRODUCTION

Valsartan[1] (VAL) chemically is N –[p-(o-1H-Tetrazol-5ylphenyl)benzyl]-N-valeryl-L-valine,is an angio tensin II receptor antagonist with particular high affinity for type 1 (AT$_1$) angiotensin receptor. Amlodipine (AMD) chemically is 2-[(2-amino ethoxy)methyl]-4-(2–chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridinedicarbooxylic acid 3-ethyl 5-methyl ester, is an antihypertensive and antianginal agent in the form of besylate salt. It is a dihydropyridine calcium-channel blocker. It is official in B.P[2].2004. Literature survey reveals estimation of valsartan in combination with other drugs by spectro-photometry in plasma[3], tablets[4] and HPLC analysis in plasma[5,6,7] while numerous methods are reported for estimation of amlodipine alone[8,9,10] while spectrophotometric[11,12], HPLC[13,14,15] and HPTLC[16,17] methods are reported for its estimation in combination

with other drugs. The present work describes two spectrophotometric methods for estimation of amlodipine and valsartan in combination.

EXPERIMENTAL
Materials
VAL and AMD Besylate was a gift sample from Alembic Pharmaceutical Ltd. Methanol AR Grade was procured from Rankem Chemicals.

Equipments
SEM and ACM was developed for VAL and AMD in their combined dosage form using Shimadzu UV 1700 double beam UV-VIS Spectrophotometer along with 1.0 cm path length matched pair of quartz cell. The absorption spectra were recorded over the wavelength

range 200-400 nm against the solvent blank. Shimadzu AUX220 balance was used for weighing the samples.

Standard Solutions

Stock solutions of VAL and AMD were prepared in methanol having concentration of 500μg/mL. Aliquots of VAL and AMD were diluted to 50.0 mL in volumetric flask to get a concentration of 10μg/mL.

Selection of Wavelength

The solutions of VAL and AMD were scanned in UV range 400-200 nm in 1.0 cm cell against blank separately. The overlain spectra (Fig. 1) showed λ_{max} for VAL at 250.0 nm and λ_{max} for AMD at 238.0 nm. The estimation of drugs by Simultaneous Equation Method (SEM) was developed using these two λ_{max} values. Similarly the estimation of Absorption correction method (ACM) was carried out at 360.0 and 236.0 nm i.e. λ_{max} values of one drug and isobestic wavelength.

Study of Beer-Lambert's law

Aliquots of working stock solution of VAL and AMD were diluted with methanol to get a concentration in 5-30 μg/mL for VAL and AMD individually and in mixture. Absorbance of each of the resulting solution was measured at 360.0, 250.0, 238.0 and 236.0 nm in 1.0 cm cell using solvent blank. The graphs were constructed as concentration vs. absorbance and correlation coefficient was found to be less than 1.

Determination of Absorptivity Value for VAL and AMD

Working standard solutions i.e. VAL 10 μg/mL and AMD 10 μg/mL were used for the present study. The procedure for preparation of both the drugs were repeated five times and absorbance of each of the solutions were measured in triplicate against solvent blank at 360.0, 250.0, 238.0 and 236.0 nm and the A (1% 1cm) values were calculated using the formula

$$A\ (1\%\ 1cm) = \frac{Absorbance}{Concentration\ (g/100\ ml)}$$

R is the absorbance ratio of AMD was calculated by following formula

Absorbance Ratio of AMD (R) =

$$\frac{Absorbance\ at\ 236.0\ nm}{Absorbance\ at\ 360.0\ nm}$$

Estimation in standard laboratory mixture

An accurately weighed quantity of VAL and AMD in the ratio of 16:1 was transferred to 50.0 mL volumetric flask separately and diluted with methanol. Aliquot portion of the above solution was further diluted to 50.0 mL with methanol to get the final concentration about 2μg/mL AMD and 32 μg/mL of VAL respectively. The absorbance of the above solution was measured at 360.0, 250.0, 238.0 and 236.0 nm against blank. The amount of VAL and AMD were calculated by substituting values in the formula below

i) Simultaneous Equation Method (SEM) / Method A

a) For estimation of AMD

$$C_x = \frac{A_1 ax_2 - A_2 ax_1}{ax_2 ay_1 - ax_1 ay_2}$$

b) For estimation of VAL

$$C_y = \frac{A_1 ax_2 - A_2 ax_1}{ax_2 ay_1 - ax_1 ay_2}$$

A_1 and A_2 are absorbances of diluted mixture at 250.0 and 238.0 nm respectively. C_x and C_y concentrations of AMD and VAL respectively (g/100 ml). ax_1, ax_2, ay_1, ay_2 are absorptivities of AMD and VAL at 250.0 and 238.0 nm respectively.

ii) Absorbance Correction Method (ACM) / Method B

a) For estimation of AMD

$$C_x = \frac{A_2}{ax_1}$$

b) For estimation of VAL

$$C_y = \frac{A_1 - (A_2 \times R)}{ax_1}$$

Where,
A1 & A2 are absorbance of diluted mixture at 236.0 nm and 360.0 nm respectively
C_x & C_y are the concentrations of AMD and VAL respectively (g/100mL); ax_1 is Absorptivity of VAL at 236.0 nm & ay_1 is Absorptivity of AMD at 360.0 nm.

Estimation of VAL and AMD in marketed formulation

An accurately weighed quantity tablet powder about 80 mg of VAL (~5 mg of AMD) was transferred to 50.0 ml volumetric flask separately and diluted with methanol, filtered through Whatmann filter paper (No.41) and aliquots were further diluted to get the final concentration of about (2 µg/ml AMD and 32 µg/ml VAL) on label claim basis. The absorbances of each of the above solutions were measured at 360.0, 250.0, 238.0 and 236.0 nm against blank. Amount of drugs were estimated by substituting the values in the equation given under Standard Laboratory mixture. Percent label claim was also determined and recorded in Table 1

Recovery Studies

It was performed by standard addition method. To the pre-analyzed powder, known quantities of VAL and AMD were added at four different levels. The contents were dissolved in methanol, filtered and the absorbances of each solution were measured at 360.0, 250.0, 238.0 and 236.0 nm against blank. Percent recovery was calculated by using the formula:

% Recovery =

$$\% \text{ Recovery} = \frac{\text{Total drug Estimated} - \text{Amt.Contributed}}{\text{Amount of Pure Drug added}} \times 100$$

The results are recorded in Table 1

VALIDATION

i) Accuracy

Accuracy was ascertained on the basis of recovery studies by standard addition method. Results were recorded in Table 1

ii) Precision

Precision of analytical method is expressed in terms of SD, %RSD of series of measurements. Study is carried out by replicate analysis of homogeneous samples of tablet powder. Results were recorded in Table 1.

Intraday Precision and Inter-day precision

An accurately weighed quantity of tablet powder equivalent to about 80 mg of VAL was diluted to get the final concentration (2µg/mL AMD and 32µg/mL VAL) on label claim basis. The absorbances of the solutions were taken after 0 hr, 3rd hr, 5th hr at selected

wavelength for intraday study. Similarly the same solution was measured on 1st, 3rd, and 5th day and % label claim was calculated. The results were recorded in Table 2.

iii) Linearity and Range

Accurately weighed quantities of tablet powder equivalent to 80, 90, 100, 110, 120% of label claim of VAL were taken and dilutions were made as described under marketed formulation. The absorbances of the resulting solutions were measured at 360.0, 250.0, 238.0 and 236.0 nm against blank. The graphs of concentration vs. absorbance were plotted and were found to be linear.

iv) Ruggedness

Different Analyst : The tablet samples were analyzed by proposed method by three different analysts and results were recorded in Table 2.

iii) Specificity

An accurately weighed quantity of tablet powder containing about 80 mg VAL (~5 mg of AMD) was transferred to 50.0 ml volumetric flask and kept under the following conditions viz., Alkali(0.1 N NaOH), Acidic(0.1 N HCl) reflux for 3 hrs, 6% H_2O_2, 3% H_2O_2 for 24 hrs (50^0C) for oxidation stress, Heat (60^0C) and humidity (75% RH) for 24 hrs, exposure to sunlight for 6 hrs and UV exposure at 254.0 nm for 24 hrs. After completion for different stress condition dilutions were made as described under marketed formulation. The absorbances of each of the above solutions were measured at 360.0, 250.0, 238.0 and 236.0 nm against blank. Results were calculated described under marketed preparation. Results are recorded in Table 3

RESULTS AND DISCUSSIONS

The overlain spectra of the VAL and AMD in methanol were recorded and λ_{max} values of both drugs and isobestic wavelength were noted. The drugs were found to obey beers law at the selected wavelength. The proposed methods method A- simultaneous equation and method B-Absorption correction method were developed and amount of each drug were calculated in laboratory mixture as well as marketed formulations. The recoveries of each drug were found to be satisfactory and within the limits. The validation of proposed methods showed that SD and %RSD values are within the limits. Hence the methods can be routinely adapted for the quality control of the drugs and is easier and statistically validated.

Table 1: Summary of the results of Marketed Formulation and Recovery Study

Sample		% Label Claim**				% Recovery*			
		Method A		Method B		Method A		Method B	
		AMD	VAL	AMD	VAL	AMD	VAL	AMD	VAL
Std.lab Mixture	Mean	100.8	100.03	99.59	99.50	------	------	-------	-------
	± SD	0.60	0.56	0.44	0.47				
	%RSD	0.60	0.56	0.45	0.47				
Formulation-I	Mean	99.76	99.37	99.80	98.82	100.13	99.56	99.75	99.48
	± SD	0.54	0.48	0.61	0.15	0.65	0.22	0.22	0.26
	%RSD	0.54	0.48	0.61	0.15	0.64	0.22	0.22	0.25
Formulation-II	Mean	100.09	99.46	99.35	99.47	100.25	99.54	100.15	99.71
	± SD	0.73	0.45	0.42	0.52	0.33	0.50	0.53	0.90
	%RSD	0.32	0.20	0.18	0.23	0.16	0.25	0.26	0.45

** Mean of five observations ± Standard Deviation (SD), Coefficient of Variation (CV), * Mean of four observations
 Method A=SEM : Simultaneous Equation Method, Method B=ACM : Absorbance Ratio Method

Table 2: Summary of validation parameters

Parameters	Percent Label Claim			
	Method A		Method B	
	AMD	VAL	AMD	VAL
Intra Day Precision (n=3)				
Amount found ±	98.66	99.42	98.29	99.34
RSD (%)	1.07	0.62	0.88	0.59
Inter Day Precision (n=3)				
Amount found ±	97.71	98.08	97.60	98.19
RSD (%)	2.01	2.10	1.56	2.02
Ruggedness (%RSD)				
Analyst to Analyst (n=3)	0.50	0.47	0.58	0.26

Method A=SEM: Simultaneous Equation Method, Method B=ACM: Absorbance Ratio Method

Table 3: Results of Specificity Study

Sample (Treated)	% Undegraded			
	Method A		Method B	
	AMD	VAL	AMD	VAL
0.1N NaOH Reflux for 3hrs.	96.52	97.03	97.17	97.43
0.1N HCl Reflux for 3hrs.	95.03	96.21	95.92	97.39
3% H_2O_2 For 24hrs at 50^0C	99.13	98.01	98.96	98.06
6% H_2O_2 For 24hrs at 50^0C	96.61	97.12	96.49	97.26
60°C For 24hrs.	92.48	95.67	96.02	93.33
Humidity (75%)	95.02	97.52	95.27	97.72
UV exposure(254.0 nm) for 24 hrs	95.80	98.43	96.94	97.98
Exposure to sunlight for 6 hrs	104.91	100.06	103.13	99.35

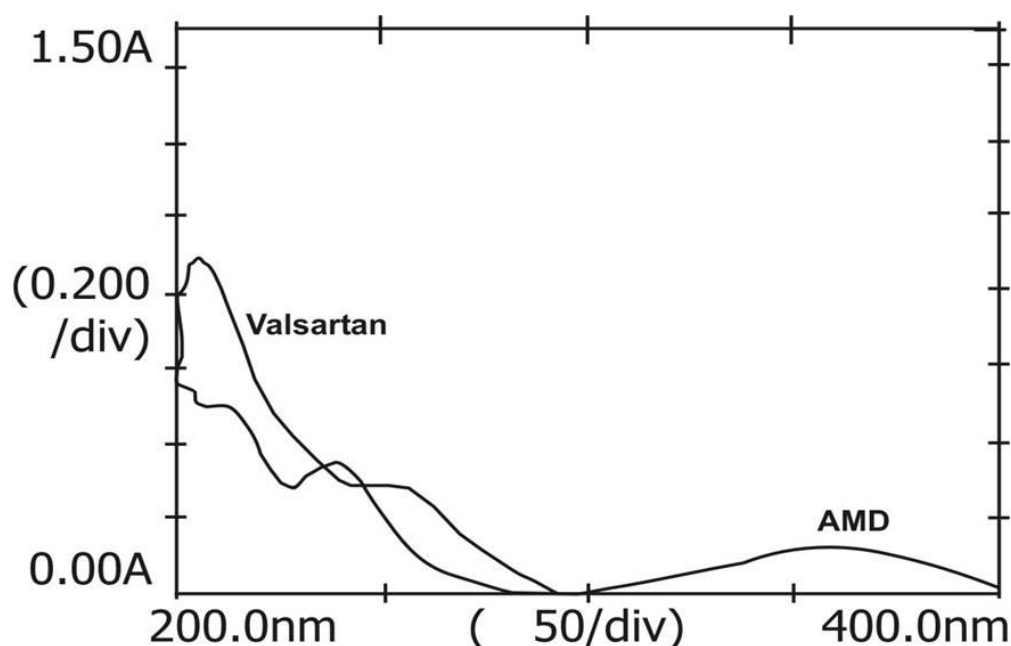Method A=SEM: Simultaneous Equation Method, Method B=ACM: Absorbance Ratio Method

Fig. 1 Overlain UV-spectrum of VAL and AMD

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