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Spectrophotometric Method Development and Validation for the Simultaneous Dissolution Determination of Amlodipine Besilate and Valsartan in Pharmaceutical Dosage Form

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ABSTRACT

A rapid, accurate, specific, linear, and sensitive spectrophotometric method has been developed and validated for the simultaneous determination of Amlodipine (Besilate) (AML) and Valsartan (VAL) dissolution in pharmaceutical dosage form ,(Valsartan/amlodipine, 160mg/10mg tablets). The method for the determination of (VAL) depends on a mathematical equation derived from the absorbance of a series of dilutions of sample stock solution and a series of AML concentrations without changing the concentration of VAL in the diluted test solution. The research work was performed on (Libra S32 PC) spectrophotometer. The linearity was performed in the concentration range of 5.0µg/ml-10.0µg/ml (AML) and 8.0-16.0 µg/ml (VAL) with a squared correlation coefficient of 0.9996 and 0.9995 for AML and VAL respectively. The Proposed methods has been validated for specificity, linearity, precision, accuracy, and all results were within the acceptance limit according to ICH guidelines and the developed method has an advantage that it can be used to calculate the percentage release of both AML and VAL from tablets by preparing test stock solution, test diluted solution and separate pure standard solution for each of AML and VAL. The method was successfully employed for routine quality control analysis in the combined pharmaceutical dosage forms.

Key words: *Amlodipine Besilate, Valsartan, Spectrophotometer, Validation.*

INTRODUCTION

Amlodipine Besilate (AML) is Calcium channel blocker. Chemically: 3-Ethyl 5-methyl (4RS)-2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-6-methyl-1,4- dihydropyridine-3,5-dicarboxylate benzenesulphonate., its molecular weight is 567.1g/mol with an empirical formula $C_{20}H_{25}ClN_2O_5,C_6H_6O_3S$. **(Fig. 1)** [1].

Valsartan (VAL) is chemically described as N-(1-oxopentyl)-N-[[2'-(1H-tetrazol-5-yl) [1, 1'-biphenyl]-4-yl] methyl]-L-valine. Its empirical formula is $C_{24}H_{29}N_5O_3$, its molecular weight is 435.5. **(Fig. 2)** [1].

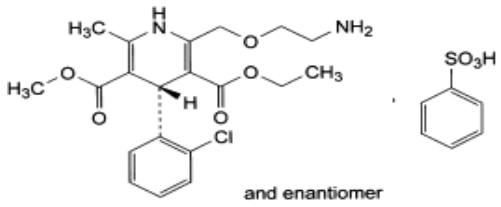

Fig. 1: Chemical Structure for Amlosipine Besilate

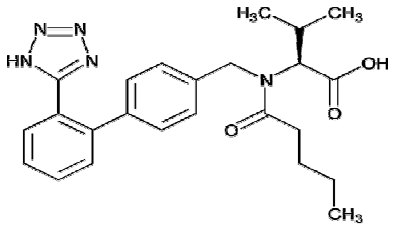

Fig. 2: Chemical Structure for Valsartan

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Literature survey reveals that few spectrophotometer methods [2-5], have been reported for the estimation of Amlodipine Besilate and Valsartan. The aim of the present study is to develop a simple, precise, linear and accurate spectrophotometric method for the estimation of Amlodipine Besilate and Valsartan dissolution from pharmaceutical dosage form.

MATERIALS AND METHODS

Instrumental and Analytical Conditions:
Reagents and Chemicals:

USP AML RS, USP VAL RS were used. AML and VAL were purchased from CADILA Pharmaceuticals and from JUBILANTS LifeSciences, respectively. Methanol was purchased from J.T. Baker, Potassium dihydrogen phosphate was purchased from Merck, Phosphoric acid was purchased from Merck and Water used was freshly prepared by Sama Pharmaceuticals Manufacturing Co.

Equipment:

A (Libra S32 PC) spectrophotometer system with Acquire Application Software was used. It was manufactured by Biochrom Ltd., UK.

Dissolution Parameters:
Medium: Phosphate buffer pH 6.8; 1000ml.
Apparatus: USP II dissolution apparatus, 75rpm, 45minutes.

Preparation of Analytical Solutions:
Preparation of Phosphate Buffer, pH 6.8:

Prepared by dissolving 47.635g of potassium dihydrogen phosphate and 6.272g of sodium hydroxide in7 liters of distilled water. The pH was adjusted to 6.8 using 5% v/v phosphoric acid or 5% w/v sodium hydroxide solutions

Preparation of standard solution for Amlodipine (Besilate) Measured at 360nm:

The Amlodipine (Besilate) standard stock solution was prepared by transferring Amlodipine Besilate equivalent to 20.0mg Amlodipine as (Besilate) standard to 200 ml volumetric flask dissolved in 10ml of methanol and the volume was completed with

dissolution medium (DM) and mixed, filtered using 0.45µ filter to obtain a solution having a concentration of 10 µg/ml.

Preparation of Valsartan standard solution Measured at 240nm:

The standard was prepared by transferring an equivalent to 16.0mg of Valsartan standard to 100 ml volumetric flask, dissolved in about 10 ml of methanol and the volume was completed with DM. 5.0 ml of the resulting solution were transferred to 50 ml volumetric flask and the volume was completed with DM to obtain a concentration of 16µg/ml of Valsartan and. Filtered through 0.45µ filter.

Preparation of the stock test solution (Valsartan and Amlodipine 160/10mg tablet Measured at 360nm:

Equivalent to 160mg valsartan, Equivalent to 10mg AML (besilate) and 179mg placebo were transferred to 1000ml volumetric flask, dissolved in 10ml of methanol to ensure complete dissolution and the volume was completed with dissolution medium (10µg/ml AML and 160µg/ml VAL).and sonicated for 20 minutes. Filtered through 0.45µ filter and measured at 360nm for AML determination.

Preparation of diluted sample solution (Valsartan and Amlodipine 160/10mg tablet:

Prepared by diluting 5ml of the stock test solution to 50 ml with DM, (1.0µg/ml AML and 16µg/ml VAL).

Spectrophotometric method Development and Validation:

The suggested analytical method was validated according to ICH guidelines with respect to certain parameters such as specificity, linearity, precision, and accuracy.

Specificity:

The specificity was carried out to determine whether there are any interference from the placebo matrix (presence of components may be unexpected to present).

Linearity:

Express ability to obtain test results where directly proportional to the concentration of analyte in the sample. The linearity of the method was established by measuring a series of standard solutions of both AML and VAL separately, the absorbance of the solutions of five different concentration levels 8.0-16.0µg/ml (VAL) and 5.0-10.0 µg/ml (AML) were measured. Construct the calibration curves for the standard solutions by plotting Absorbance against respective concentrations. Linear regression was applied and slope-a, intercept-b, and squared correlation coefficient-R^2 were determined.

Precision:

Express the closeness of agreement between the series of measurement obtained from multiple sampling of same homogeneous sample under the prescribed conditions.

In order to determine precision, six independent sample solution preparations from a single lot of formulation 10µg/ml for AML and 160µg/ml for VAL tested, expressed as mean and %RSD calculated from the data obtained which are found to be within the specified limits.

Accuracy:

Accuracy was determined in terms of percentage recovery. The accuracy study was performed for 50%, 70% and 100 % for AML and VAL. The absorbance of the standard and sample solutions were measured in triplicate and percentage recoveries of AML and VAL were calculated. The absorbance of each level was used for calculation of % recovery.

RESULTS AND DISCUSSION

The present investigation reported is a new spectrophotometric method development and validation of simultaneous estimation of AML and VAL. The method developed was proceeding with wavelength selection. The optimized wavelength was 240nm for VAL and 360nm for AML.

The method for the determination of (VAL) depends on the ratios of the deference (DEFs) between the absorbance of the10 folds diluted {(VAL/AML diluted test solution) and the (VAL) pure standard solution at 240nm} to the absorbance of the (AML) test solution at 360nm. A series of (AML and VAL) test solutions(5µg/ml AML and 160µg/ml VAL, 6µg/ml and 160µg/ml VAL , 7µg/ml and 160µg/ml VAL, 8µg/ml and 160µg/ml VAL, 10µg/ml and 160µg/ml VAL were prepared and their absorbance measured at 360nm and A series of (AML and VAL) mixtures test solutions(0.5µg/ml AML and 16µg/ml VAL, 0.6µg/ml and 16µg/ml VAL, 0.7µg/ml and 16µg/ml VAL, 0.8µg/ml and 16µg/ml VAL, 1.0µg/ml and 16µg/ml VAL were prepared and their absorbance measured at 240nm . A pure AML standard solution was prepared at a concentration of 10µg/ml and measured at 360nm and a pure VAL standard solution was prepared at a concentration of 16µg/ml and measured at 240nm. For the determination the percentage of (VAL) released the absorbance of the test solutions measured at 360nm were plotted against the (DEFs) measured at 240nm. The equation was found and used for the determination of the percentage released of valsartan.

At 360 nm the VAL in the stock test solution did not show any absorbance and the percentage release of the AML measured directly from the concentration of AML pure standard solution. The specificity of the method was to determine whether there are any interference. The linearity was determined as linearity regression of the claimed analyte concentration of the range 5.0-10µg/ml (AML) and 8.0-16.0µg/ml (VAL). The calibration curve obtained by plotting Absorbance of versus concentration and presented in **Table 1** was linear and the squared correlation coefficient was found to be 0.9996 and 0.9995 for AML and VAL respectively. The precision of the method was ascertained from determinations of absorbance of six replicates of sample solution. The %Relative Standard Deviation for method precision presented in **Table 2** was found to be 0.635 and 0.32 for AML and VAL respectively.

The accuracy study was performed in 50%, 70% and 100% .The percentage recovery was determined for VAL and AML and was found to be 100.4% and 99.8% presented in **Tables 3& 4**.

Table No. 1: Linearity results for Amlodipine (Besilate) and Valsartan

Amlodipine (Bisilate)		Valsartan	
Absorbance	**Concentraion (µg/m)**	**Absorbance**	**Concentraion (µg/m)**
0.078	5	0.244	8.0
0.092	6	0.300	9.6
0.108	7	0.351	11.2
0.124	8	0.399	12.8
0.154	10	0.500	16.0

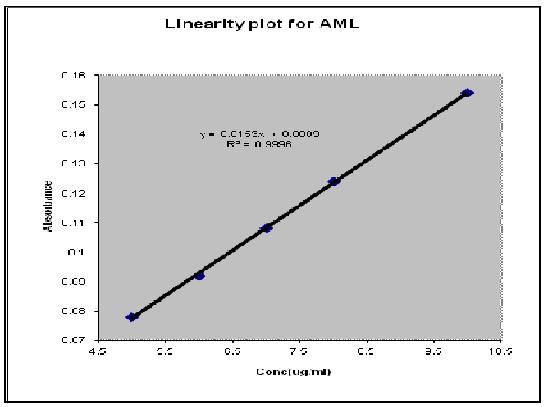

Fig. 3: Linearity plot for AML

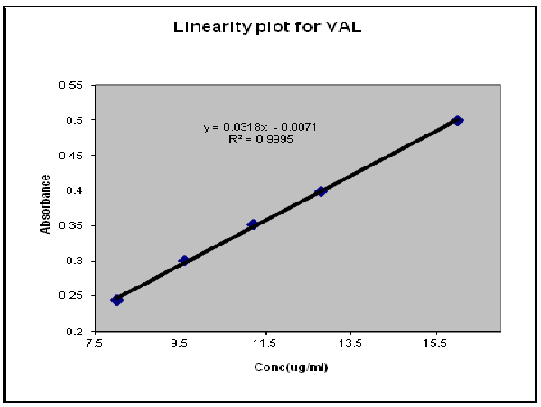

Fig. 4: Linearity plot for VAL

Table No. 2: Precision results for Amlodipine (Besilate) and Valsartan

Determination	AML	VAL
	Absorbance	Absorbance
1	0.156	0.501
2	0.154	0.501
3	0.154	0.501
4	0.156	0.500
5	0.154	0.501
6	0.155	0.497
Average	0.155	0.500
Std. Dev.	0.001	0.002
RSD%	0.635	0.320

Table No. 3: %Recovery for VAL (Average of 3 determinations at each level)

Concentraion (at Specific Level)	Active Druge adding in mg	Recovered Amount in mg	Mean Recovery
50%	**80**	**80.4**	**100.40%**
70%	**112**	**112.9**	
100%	**160**	**160.16**	

Table No. 4: %Recovery for AML (Average of 3 determinations at each level)

Concentraion (at Specific Level)	Active Druge adding in mg	Recovered Amount in mg	Mean Recovery
50%	**5**	**4.957**	**99.80%**
70%	**7**	**6.95**	
100%	**10**	**10.04**	

Calculations:

Formula (1) for the calculation of % Release of Valsartan (%RV):

%RV = [A (ts 240nm)-{(0.238* A (ts360nm) +0.002}]*(100/ A (VAL sd 240nm)*(formula 1)*

Formula (2) for the calculation of % Release of Amlodipine:

% Release of Amlodipine = (A (ts360nm)/A (AML sd 360nm)) *100 *(formula 2)*

Where:

A (ts 240nm) = Absorbance of diluted test solution at 240nm

A (ts360nm) = Absorbance of stock test solution at 360nm

A (VAL sd 240nm) = Absorbance of pure VAL standard solution at 240nm

A (ts360nm) = Absorbance of stock test solution at 360nm

A (AML sd 360nm) = Absorbance of pure AML standard solution at 360nm

SUMMERY

The method was found to be precise accurate and linear for determination of Amlodipine Besilate and Valsartan. The method was developed and validated for linearity, range, specificity and accuracy. All parameters tested were found to be within limits. The study indicates that the method has significant advantages in term of shorter analysis time, specificity, accuracy and precision.

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