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NOVEL VALIDATED UV SPECTROSCOPIC METHOD FOR THE ANALYSIS OF AMLODIPINE BESYLATE AND VALSARTAN IN DRUG SUBSTANCE AS FIXED DOSAGE FORM

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ABSTRACT

For the analysis of amlodipine besylate and valsartan in drug substance as a fixed dosage form, a simple, valid, exact, and contemporaneous UV spectrophotometric approach was devised. The quantitative measurement of mixed dosage of amlodipine besylate is 3-Ethyl 5-methyl2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydro-3,5-pyridinedicarboxylate and valsartan is (2S)-3-methyl-2-[pentanoyl-[[4-[2-(2H-tetrazol-5-yl) phenyl] phenyl] methyl] amino] butanoic acid are done by using UV spectroscopy. UV spectroscopy is based on the absorption of visible light or UV light by pharmacological molecules. Normally, the research of UV spectroscopy follows Beers Lambert's rule, therefore Q analysis was used to estimate the (AUC) area under curve for amlodipine besylate and the standard curve for valsartan at the same time. Amlodipine besylate has a lambda max of 240 nm, while valsartan has a lambda max of 250 nm. Determination is done by immersing the mixed dosage in the methyl alcohol and calibration. Following this step, it undergoes validation processes such as linearity, range, accuracy, precision, LOD (Loss of detection), LOQ (Loss of quantification), assay, stability parameter studies, and regression value is also found. Hence, this study explains about the quantitative estimation of amlodipine besylate and valsartan by UV spectroscopy method for the identification of trace metals. Additionally, validation of test processes is done to verify the potency of drug substances and also reveals the potency of fixed oral dosage form.

Keywords: Amlodipine besylate, Valsartan, UV Spectrophotometry, Validation, Fixed dose

INTRODUCTION

Combination therapy is when two or three drugs are combined together. About 1.15 million people worldwide suffer from hypertension. According to WHO, the highest number of hypertension cases are seen in Africa (27%) and least number of hypertension cases are seen in America (18%) **[1-2]**. In general, adults are more affected by hypertension. Due to a change in lifestyle, pollution and food habits, there is an increase in stress levels among people. High levels of stress causes elevated blood pressure which could lead to further complications that may be life-threatening **[3-4]**. It is inconvenient for patients to take two or more drugs at a time, so the US FDA approved Amlodipine besylate and Valsartan as a single pill.

Chemically, amlodipine besylate is 3-Ethyl 5-methyl 2-[(2-aminoethoxy) methyl]. -4-(2-chlorophenyl) -6-methyl-1,4-dihydro-3,5-pyridinedicarboxylate,it is a calcium channel blocker and used to treat cardiac vascular disorders by enhancing blood flow **[5, 6]**. Amlodipine besylate is a synthetic dihydropyridine with antihypertensive and antianginal properties **[7]**. Amlodipine prevents calcium ions from accessing cardiac and vascular smooth muscle cells and as a result, the primary coronary and systemic arteries dilate leading to reduced contractility of the myocardium, enhanced blood circulation and supply of oxygen to cardiac tissue, and reduction of total peripheral resistance. Through suppression of the p-glycoprotein efflux pump, this substance may also affect multi-drug response (MDR) activity. Amlodipine besylate's physicochemical features include a melting point of 109°C and the fact that it is soluble in water. Amlodipine besylate has a refractive index of 1.731 **[8- 10]**.

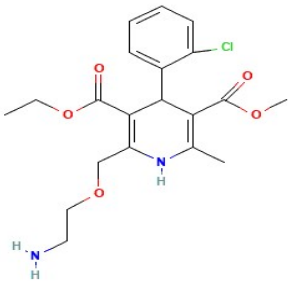

Figure 1: Structure of Amlodipine besylate

Valsartan is a nonpeptide triazole-derived antagonist of angiotensin (AT) II with antihypertensive effects that is chemically (2S)-3-methyl-2-[pentanoyl-[[4-[2-(2H-tetrazol-5-yl) phenyl] phenyl] methyl] amino] butanoic acid. Valsartan inhibits the action of the AT II, Synthesis and secretion of aldosterone, and sodium reabsorption in the kidney, resulting in vasodilation, increased sodium and water excretion, a decrease in plasma volume and a lowering of blood pressure. Valsartan has a melting point of 107°C and is soluble in ethanol and methanol, according to its physicochemical parameters. It has a refractive index of 1.587 **[11-13]**.

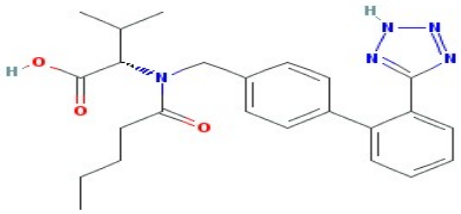

Figure 2: Structure of Valsartan

The combined formulation of amlodipine besylate and valsartan is not altered by food and the maximum peak plasma concentration in the oral dosage form is reached at 3, 6 and 8 hrs respectively. The oral formulation of amlodipine besylate and valsartan results in lowering of blood pressure, which undergoes 8 to 16 clinical trials for further clarification. On the whole amlodipine besylate and Valsartan is well tolerated in clinical trials. The quantitative estimation of amlodipine besylate and valsartan by (Spectroscopy in the ultraviolet range) approach for trace metal detection is discussed in this paper.

EQUIPMENTS USED

S. No.	Name of the equipment	Company
1.	UV 3092 UV/Visible spectrophotometer with 1 cm matched Quartz cells.	LABINDIA
2.	Electronic Balance	Shimadzu BL-220H, Japan
3.	LI 120 PH Meter	Elico India.
4.	R8c Laboratory Centrifuge	Remi motors Ltd, India
5.	Vortex Mixer	Remi motors Ltd, India.
6.	Ultra-Sonicator	ILE

STANDARD STOCK SOLUTION PREPARATION

Meticulously weighed and transferred into 100 milliliter standard flasks of 100 milligram of each drug valsartan and Amlodipine in two separate standard flasks and dissolved in methyl alcohol to get the strength of 1 milligram/milliliter of each. From the stock, 1milliliter is pipette out and reduced the strength of working standard solution 10microgram per milliliter of both drugs with methyl alcohol.

λ MAX DETERMINATION

Both Valsartan and Amlodipine besylate standard concentration were prepared of dilutions 10microgram per milliliter both the solutions were scanned in UV range against blank solvent. The wavelength spectra of Valsartan and Amlodipine besylate in methyl alcohol are shown in **Figure 3 and 4** respectively. The representative of each spectrum revealed that Valsartan shows a λmax at 250 nanometer and Amlodipine besylate shows at 240 nanometer.

PREPARATION OF CALIBRATION CURVE

Valsartan and Amlodipine besylate working standard solutions were prepared of dilution 100µg/milliliter. Different dilution was taken from standard stock solution and diluted with methyl alcohol in

the concentration of 2 microgram/milliliter to 12 microgram/milliliter solutions at 2 microgram/milliliter interval. The working solutions of Valsartan and Amlodipine were prepared and scanned at 250 nanometer and 240 nanometer respectively. The absorbances were recorded and are outlined against the strength to obtain the respective calibration curves **(Figure 3 and Figure 4)**.

LINEARITY AND RANGE

For the estimation of Amlodipine besylate and Valsartan lambda max were found to be for Amlodipine besylate 240 nanometer and for Valsartan was found to be 250 nanometer in methyl alcohol solvent. The linearity for both amlodipine besylate and Valsartan in the strength range of 2-12 microgram/milliliter **(Table 1 and Figure 5 and 6)**.

PRECISION

For the intra-day calculation of precision 0-10 hr with the interval of every two hr and interday precision 1-6 days were chosen and readings were taken for every day for Amlodipine besylate and Valsartan tabulated in **Table 2**.

STABILITY PARAMETER

The precise amount of tablet formulation which is equal to 2.5 milligram of Amlodipine besylate and 2.5 milligram of Valsartan was transferred into 100 milliliter standard flask and maintained under the subsequent conditions which hold Alkaline (0.1 N Sodium hydroxide), Acidic (0.1 N Hydrochloric acid) reflux for 3 hr, 3% Oxydol at 50°C, heat (60°C), humidity (75 percentage Relative humidity) for 24 hr and after the particular time quantity was made up to the mark with distilled water, separated using Filter paper. From this stock solution, 5 milliliter portion of the filtrate was pipetted out and further diluted with distilled water in a 100 milliliter standard flask (10 microgram/milliliter). The standard stock solution of two drugs were prepared and compared against a label claim and results were tabulated in **Table 3**.

DETECTION LIMIT AND QUANTIFICATION LIMIT

The detection limit (LOD) and quantification limit (LOQ) for Amlodipine besylate verified to be 0.15 microgram/milliliter and 0.32 microgram/milliliter respectively. The detection limit (LOD) and quantification for Valsartan examined to be 0.35 microgram/milliliter and 0.95 microgram/milliliter **(Table 4)** respectively.

ACCURACY

Accuracy was determined for drugs by spiking with 80, 100 and 120 percentage of pure drug and the mean recovery of the Amlodipine besylate and Valsartan were to be 99.58% and 99.51% respectively **(Table 5)**.

ASSAY

The assay of Amlodipine besylate and Valsartan were done and its percentage purity found to be 99.95% and 96% respectively.

RESULTS AND DISCUSSION

Combination of Amlodipine besylate and Valsartan:

The extent of Amlodipine besylate another Valsartan bulk samples and their tablet forms were determined by simultaneous equation method by using UV Spectrophotometer. The regression strength of Amlodipine besylate and Valsartan over its absorbance's were obtained as y=0.051x-0.01066 and y=0.055x-0.005 respectively with a correlation coefficient (r2) of 0.998 for Amlodipine besylate and 0.999 for Valsartan. The intra-day precision in addition inter-day precision for Amlodipine besylate and its % RSD were obtained as 0.15% and 0.25% respectively. The intra-day precision in addition inter-day precision for Valsartan and % RSD were obtained as 0.16% and 0.24% respectively. This confirms the procedure is precise. Accuracy is determined for both drugs by spiking with 80, 100 and 120% of additional pure drug and the % mean recovery of the Amlodipine besylate and Valsartan were obtained as 99.58 and 99.51 respectively **(Table 5)**. The percentage purity for the assay of Amlodipine besylate and Valsartan were obtained as 96 and 99.95 respectively **(Table 6)**. The assay result shows that the methodology was selective for evaluation of Amlodipine besylate and Valsartan without hindering from the inactive substance used in tablet dosage form.

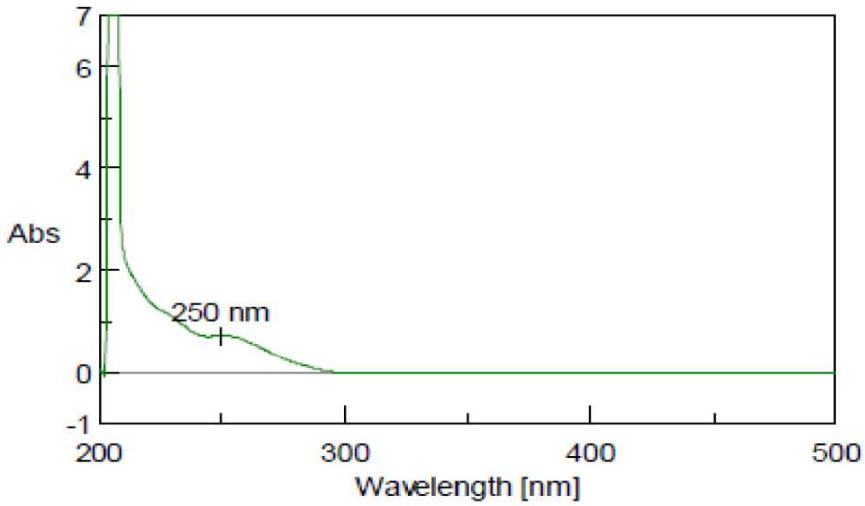

Figure 3: Chromatogram of Valsartan

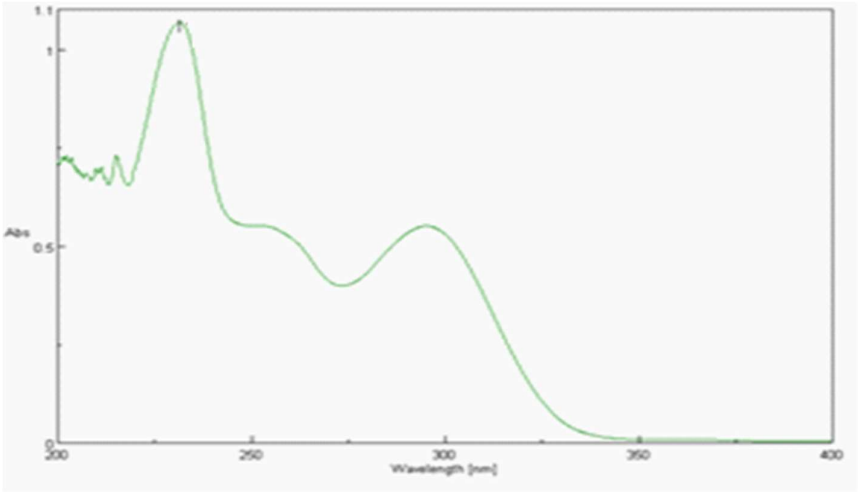

Figure 4: Chromatogram of Amlodipine besylate

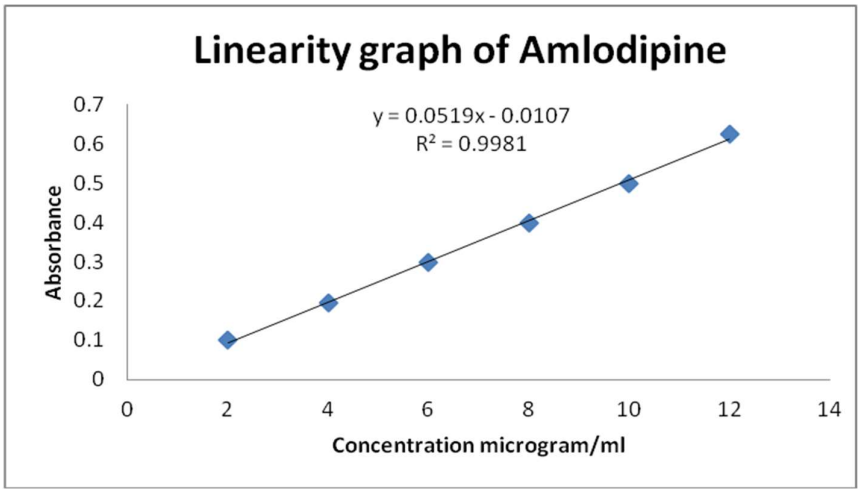

Figure 5: Linearity graph of Amlodipine besylate

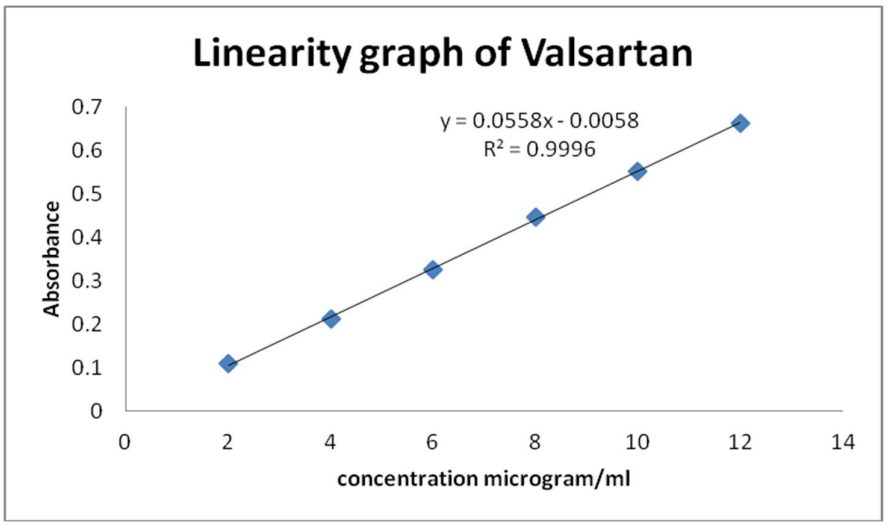

Figure 6: Linearity graph of Valsartan

Table 1: Linearity datas for Amlodipine besylate and Valsartan

S. No.	Concentration(µg/mL)	Amlodipine besylate Absorbance	Valsartan Absorbance
1	2	0.0994	0.1099
2	4	0.1955	0.2119
3	6	0.2997	0.3254
4	8	0.3984	0.4464
5	10	0.4978	0.5512
6	12	0.6248	0.6631
Slope		0.051	0.055
Intercept		-0.01066	-0.005
Regression Equation(y)		0.051x - 0.01066	0.055x - 0.005
Correlation Coefficient		0.998	0.99

Table 2: Intra-day and Inter-day precision results of Amlodipine besylate and Valsartan

Intra-day precision				Inter-day precision		
S. No.	Time (Hours)	Amlodipine Absorbance	Valsartan Absorbance	Time (Days)	Amlodipine Absorbance	Valsartan Absorbance
1	0	0.503	0.546	1	0.506	0.545
2	2	0.502	0.542	2	0.504	0.543
3	4	0.501	0.540	3	0.502	0.544
4	6	0.502	0.544	4	0.501	0.546
5	8	0.502	0.545	5	0.501	0.546
6	10	0.503	0.546	6	0.502	0.547
Mean		0.502	0.543	Mean	0.502	0.545
SD		0.000753	0.00089	SD	0.00013	0.0013
%RSD		0.15	0.16	%RSD	0.25	0.24

Table 3: Stability studies parameters for Amlodipine besylate and Valsartan

Sample (treated)	Percent Label claim	
	Amlodipine besylate	Valsartan
0.1 N NaOH	98.67	99.11
0.1 N HCl	95.19	95.92
60°C for 2hr	98.79	99.01
Humidity (75% RH)	95.97	96.52

Table 4: LOD and LOQ of Amlodipine besylate and Valsartan

Parameter	Amlodipine besylate measured value(µg/mL)	Valsartan measured value(µg/mL)
Limit of detection	0.10	0.31
Limit of quantification	0.32	0.95

Table 5: Recovery studies for Amlodipine besylate and Valsartan

Amlodipine besylate				Valsartan		
	80%	100%	120%	80%	100%	120%
Std. conc. (microgram/milliliter)	10	10	10	10	10	10
Conc. added (microgram/milliliter)	8	10	12	8	10	12
Conc. found (microgram/milliliter)	7.96	9.95	11.97	7.94	9.98	11.94
% Recovery	99.5	99.5	99.75	99.25	99.8	99.5
% Mean recovery	99.58			99.51		

Table 6: Assay of Amlodipine besylate and Valsartan formulations

Formulation		Label claim	Amount found	% Assay
Valzaar SM	Valsartan	80mg	79.96mg	99.95
	Amlodipine besylate	2.5mg	2.4mg	96

CONCLUSION

From the above discussion, the proposed UV spectroscopy method were found to be facile, precise, specific, accurate and economical for the analysis of Amlodipine besylate and Valsartan in drug substance a fixed dosage form. The assay results for Amlodipine besylate and Valsartan in fixed dosage form by the UV spectroscopy method shows no significant changes from

the inactive substance used in tablet dosage form. The validation data and recovery studies indicate the method is free from intercede from the inactive substance.

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CONFLICT OF INTEREST

The authors declare no conflict of interest

ABBREVIATIONS

UV: Ultraviolet-Visible Spectroscopy; **µg:** Microgram; **mL:** Milli Liter; **mg:** Milligram;

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