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Simultaneous estimation of amlodipine and losartan by UV-method in bulk drug and tablet dosage formulation

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ABSTRACT

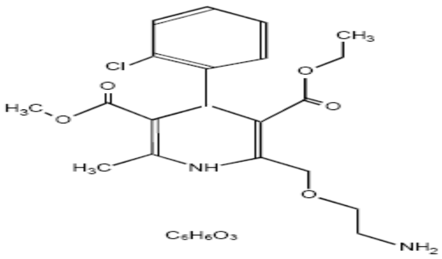
The present study describes simple, accurate, precise and cost effective UV Spectrophotometric method for the simultaneous estimation of Amlodipine besylate (AMD) and Losartan potassium (LP) in bulk and tablet dosage form. The solvent used was methanol and the absorption maxima for amlodipine besylate and losartan potassium were found to be 237nm and 202nm respectively. A linear response was observed in the range of 1.25-7.5µg/mL and 12.5-75µg/mL with a correlation coefficient of 0.99 for AMD and LP respectively. The method was then validated for different parameters like accuracy, precision, sensitivity and linearity as per ICH Q2 (International Conference on Harmonization) guidelines. This method can be used for the simultaneous estimation of AMD and LP in quality control of formulation without interference of excepients.

Key Words: Amlodipine besylate, Losartan potassium, UV-Spectrophotometry and method development.

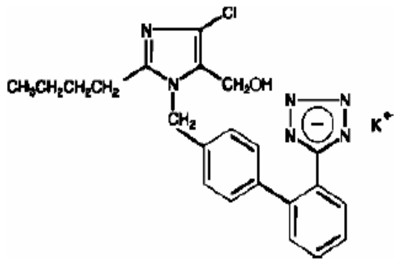
INTRODUCTION

Amlodipine besylate (AMD) is chemically -[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridinedicarboxylic acid,3-ethyl,5-methyl ester besylate mono benzene sulphonate, is a dihydropyridine calcium antagonist (calcium ion antagonist or slow-channel blocker) that inhibits the transmembrane influx of calcium ions into vascular smooth muscle and cardiac muscle. Experimental data suggest that amlodipine binds to both dihydropyridine and nondihydropyridine binding sites. The contractile processes of cardiac muscle and vascular smooth muscle are dependent upon the movement of extracellular calcium ions into these cells through specific ion channels. Amlodipine inhibits calcium ion influx across cell membranes selectively, with a greater effect on vascular smooth muscle cells than on cardiac muscle cells.[1,2,3]

Losartan potassium (LP), or (2-butyl-4-chloro-1-{[2'-(1*H*-tetrazol-5yl)biphenyl]methyl}-1*H*-imidazol-5-yl) methanol monopotassium salt, is a selective, competitive angiotensin II receptor type 1 (AT_1) receptor antagonist, reducing the end organ responses to angiotensin II. Losartan administration results in a decrease in total peripheral resistance (after load) and cardiac venous return (preload).[3,4]



Amlodipine besylate



Losartan potassium

From literature survey it was found that many methods had been reported for determination of amlodipine besylate [5,6,7,8,9,10] and losartan potassium [11,12,13,14] individually but in combination not many methods have been reported so far. In this present research work, it was proposed to develope and validate a new, simple and accurate UV method for simultaneous estimation of Amlodipine besylate and Losartan potassium in marketed dosage formulations.

MATERIALS AND METHODS

The instrument used for the study was a UV-Visible spectrophotometer (SHIMADZU UV-1800 240V) having two matched quartz cells with 1cm path length. The solvent used was methanol (AR grade).

2.1 Method Development:

2.1.1 Solubility test: Solubility test for AMD and LP was performed by using various solvents. Both the drugs were freely soluble in methanol and practically insoluble in water. Hence methanol was selected as a solvent for the proposed method.

2.1.2 Determination of Absorption maxima:

Standard solution of Amlodipine Besylate and Losartan Potassium:

Accurately weighed 5 mg of amlodipine besylate and 50 mg of losartan potassium was transferred into clean, dry 50 mL volumetric flask separately and dissolved with sufficient volume of methanol. The volume was made up to 50 mL with methanol to get concentrations of 100µg/mL and 1000µg/mL. 0.5 mL of the above solution was further diluted to 10 mL to get the concentration of 5µg/mL and 50µg/mL.

Determination: Working standard solutions of both the drugs individually and in combination were scanned in the UV range of 200 to 400 nm, using methanol as blank. AMD and LP were showed good absorption at 237nm and 202nm respectively. Isobestic point for the combination was found to be 230nm.

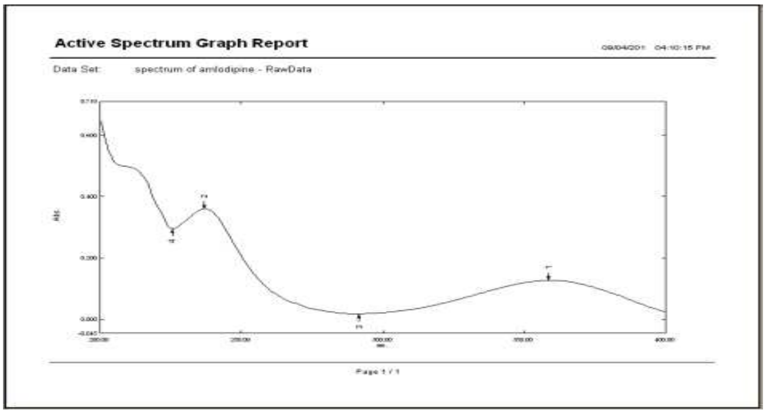


Fig 1: UV-Spectrum of AMD

2.1.3 Determination of Absorptivity

Standard stock solution of Amlodipine besylate and losartan potassium:

Accurately weighed 5 mg of Amlodipine Besylate and 50 mg of losartan potassium was transferred into a clean, dry 50 mL volumetric flask separately and dissolved with sufficient volume of methanol. The volume was made up to 50 mL with methanol to get concentrations of 100µg/mL and 1000µg/mL. 5 mL of the above solutions was further diluted to 50 mL to get the concentration of 10µg/mL and 100µg/mL.

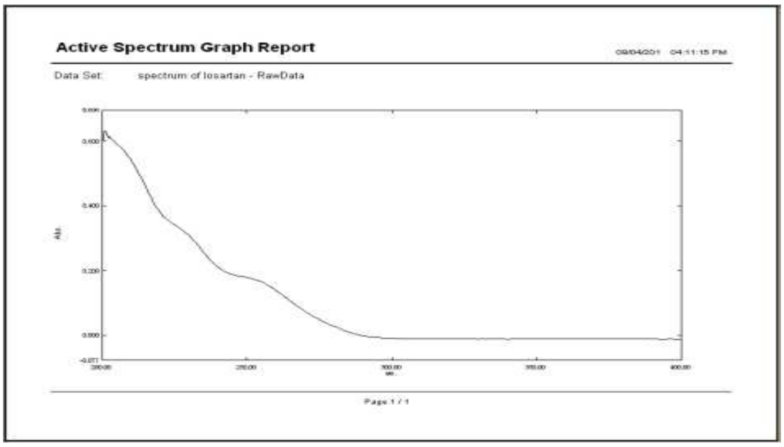


Fig 2: UV-Spectrum of LP

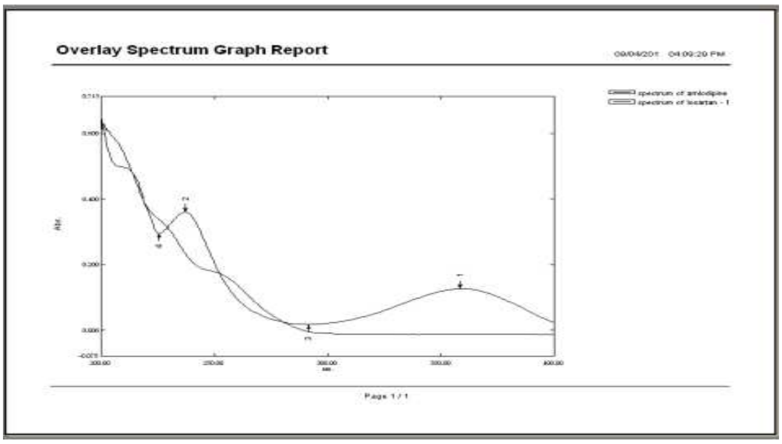


Fig 3: Overlay Spectrum of AMD and LP

Working standard stock solution:
The stock solution of volumes 1.25, 2.5, 5, 6.25 and 7.5 mL was further diluted in separate 10 mL volumetric flasks with methanol to get the concentrations of 1.25, 2.5, 5, 6.25 and 7.5µg/mL for amlodipine besylate and 12.5, 25, 50, 62.5 and 75µg/mL for losartan potassium respectively.

Propcedure:
The absorbances of both the drugs were recorded at 237 and 202 nm and molar absorptivity (€) for both the drugs was calculated from the formula:

$$€ = A/C$$

Where, A = absorbance,
C = concentration of analyte in µg/100mL.

Table 1: Absorptivity data for AMD and LP

SI. No	Conc. (µg/ml)	Amlodipine Besylate				Conc. (µg/mL)	Losartan potassium			
		A_{x1}	A_{x2}	$€_{x1}$	$€_{x2}$		A_{y1}	A_{y2}	$€_{y1}$	$€_{y2}$
1	1.25	0.046	0.035	368	280	12.5	0.523	0.58	418.4	464
2	2.5	0.054	0.046	216	184	25	0.633	0.669	253.2	267.6
3	5	0.068	0.057	136	114	50	0.783	0.796	156.6	159.2
4	6.25	0.075	0.068	120	108.8	62.5	0.835	0.892	133.6	142.72
5	7.5	0.083	0.078	110.66	104	75	0.921	0.992	122.8	132.26
Average				**190.13**	**158.16**				**121.92**	**233.15**

2.1.4 Development of Simultaneous Equation :
If sample contains two absorbing substance (X and Y) and each of which absorbs at the Wavelength maxima of the other. Then it is possible to determine both the drugs by the technique of simultaneous Equation.

The information required is:
λ_1: Wavelength maxima for Amlodipine besylate
λ_2: Wavelength maxima for Losartan potassium
a_{x1} and a_{x2}: Absorptivity of Amlodipine besylate at 237 nm and 202 nm
a_{y1} and a_{y2}: Absorptivity of Losartan potassium at 237 nm and 202 nm
A_1: Absorbance of Amlodipine besylate at 237 nm
A_2: Absorbance of Losartan potassium at 202 nm

Let C_X and C_Y be the concentration of Amlodipine besylate and Losartan potassium respectively in the diluted sample:

$$C_x = (A_2 a_{y1} - A_1 a_{y2}) / (a_{x2} a_{y1} - a_{x1} a_{y2})$$
$$C_y = (A_1 a_{x2} - A_2 a_{x1}) / (a_{x2} a_{y1} - a_{x1} a_{y2})$$

2.1.5 Assay of tablet formulation:

20 tablets were weighed; the powder equivalent to 5 mg of amlodipine besylate and 50mg of losartan potassium was accurately weighed and transfered into clean, dry 100mL volumetric flask. The powder was first dissolved in few mL of methanol by sonication, the volume was made up to 100mL and then filtered through a Whatmann filter to obtain the concentrations of 100µg/mL and 1000µg/mL for amlodipine besylate and losartan potassium respectively. From the above stock, 0.5mL was transferred into a 10mL volumetric flask and volume made up to 10mL with the methanol to get the concentrations of 5µg/mL for amlodipine besylate and 50µg/mL for losartan potassium respectively.

Determination: Absorbance of working sample solutions at 237nm and 202nm was recorded as A1 and A2. Using simultaneous equation method the concentration of Amlodipine besylate and Losartan potassium was calculated in serial concentrations.

Table 2: Assay of Tablet dosage form

Drug	Labelled amount(mg)	Amount found	%Label claim
AMD	5	4.58	99.08
LP	50	50.09	100.06

2.2 Method Validation [15]:

Validation of an analytical method is the process to establish that the performance characteristics of the developed method meet the requirements of the intended analytical application. The UV method was validated in terms of accuracy, precision, LOD, LOQ, linearity and sensitivity.

2.2.1 Accuracy

This parameter is performed to determine the closeness of test results with that of the true value which is expressed as % recovery. These studies were performed at three different levels (50%, 100% and 150%) and the % recovery of amlodipine besylate and losartan potassium was calculated.

Table 3: Recovery data of AMD and LP

Drug	Levels	Mean recovery (%)	±SD	% RSD
AMD	**L_1**	100.29	0.01	1.61
	L_2	100.8	0.008	0.90
	L_3	98.2	0.35	0.35
LP	**L_1**	99.3	0.04	0.51
	L_2	100	0.13	0.83
	L_3	102.3	0.13	0.13

2.2.2 Precision

The precision (system, method) of the proposed method was evaluated by carrying out six independent assays of test sample. RSD (%) of six assay values obtained was calculated. The intermediate precision was carried out by analyzing the sample in different days.

Table 4: Intermediate precision data of AMD and LP

Compound	Intra-day precision		Inter-day precision	
	Conc.	% RSD	Conc.	% RSD
AMD	5.23	0.37	5.99	0.33
LP	66.2	0.41	60.97	0.52

2.2.3 Limit of Detection and Limit of Quantitation

The limit of detection (LOD) and the limit of quantitation (LOQ) for amlodipine besylate and losartan potassium were determined from the visualization method. In visualization method lower dilutions of the standard drugs were made, and absorbance was recorded.

Table 5: Data of LOD & LOQ for AMD

Volume of Amlodipine Besylate stock solution	Volume made up to(mL)	Concentration (µg/mL)	Absorbance
0.7	10	0.07	0.027
0.5	10	0.05	0.013
0.3	10	0.03	0.007
0.2	10	0.02	0.004
0.1	10	0.01	_

Table 6: Data of LOD & LOQ for LP

Volume of Losartan potassium stock solution	Volume made up to(mL)	Concentration (µg/mL)	Absorbance
1	10	0.1	0.046
0.7	10	0.07	0.032
0.5	10	0.05	0.021
0.3	10	0.03	0.009
0.1	10	0.01	_

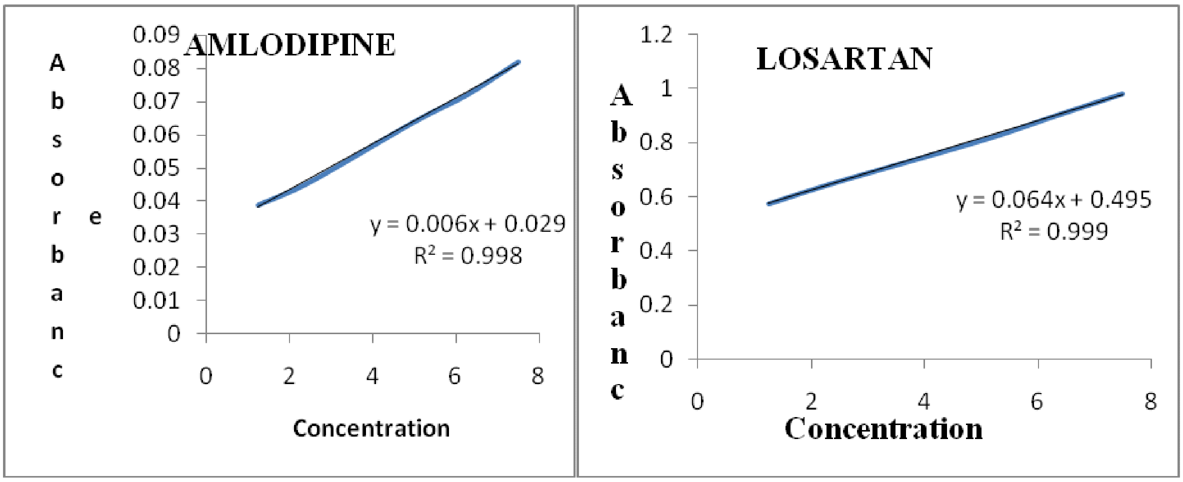
2.2.4 Linearity:

Linearity was evaluated through a linear regression analysis. The linearity for AMD (1.25-7.5µg/mL) and LP (12.5-7.5µg/mL) were determined in terms of correlation coefficient.

Table 7: Linearity data for AMD and LP

Linearity (n=5)	AMD	LP
Range	1.25-7.5µg/mL	12.5-75µg/mL
Mean ‘r^2’ value	0.998	0.999
Regression equation	Y=0.0069x+0.0296	Y=0.0643x+0.495

Fig 4: Linearity graph of AMD and LP



2.2.5 Sensitivity:

The sensitivity depends upon the experimental conditions. The maximum sensitivity of which a method is capable is expressed in terms of detection limits. Knowledge of sensitivity of the reaction is important and an easily detectable change in intensity must be obtained by small changes in the concentration. In this manner very small amounts of the constituents can be determined. The sensitivity is expressed as Sandell’s sensitivity (п) and calculated with formula.

$$\Pi\ (\mu g/cm^3/AU) = \frac{\text{Conc.of drug } (\mu g/100mL)}{\text{Absorbance}} \times 0.001$$

Table 8: Sensitivity data for AMD and LP

Conc.	Amlodipine Besylate		Conc.	Losartan Potassium	
	Absorbance at 237 nm	Sandell Sensitivity		Absorbance at 202 nm	Sandell Sensitivity
1.25	0.042	2.97	12.5	0.596	2.09
2.5	0.049	5.10	25	0.661	3.78
5	0.065	7.69	50	0.798	6.26
6.25	0.073	8.56	62.5	0.906	6.89
7.5	0.083	9.03	75	0.975	7.69
Avg		**6.67**	**Avg**		**5.34**

Force degradation study[16]:
Both the drugs AMD and LP were subjected to stress testing as per ICH recommended test conditions. The drugs were subjected to acid hydrolysis by using 0.1N hydrochloric acid and alkali hydrolysis by using 0.1N sodium hydroxide solution; oxidation by using 30%v/v solution of hydrogen peroxide; thermal and photolysis. The objective of stress study was to generate the degradation products under various stress conditions.

Table 9: Degradation results of Amlodipine and Losartan

S.No	Absorbance		Concentration		% Assay	
	AML	LP	AML	LP	AML	LP
Control*	0.064	0.819	5.02	50.62	100.29	101.25
Acidic*	0.063	0.807	4.88	48.75	97.35	97.5
Oxidation*	0.064	0.809	5.02	49.06	100.29	98.12
Thermal*	0.064	0.815	5.02	50	100.59	100
Alkali*	0.063	0.805	4.88	48.43	97.35	96.87

RESULTS AND DISCUSSION

The calibration plot for the method was linear over the concentration range of 1.25-7.5µg/mL for AMD and 12.5-75µg/mL for LP respectively. The determination of coefficients (r^2) was 0.998 and 0.999 for AMD and LP respectively. The method was found to be precise and as the %RSD values for intra day and inter day were found to be less than 1% for AMD and LP respectively. % recovery (97.3-102.3%) was found to be good at each added concentration, indicating that method was accurate. The LOD and LOQ were found to be 0.02µg/ml and 0.03µg/ml for AMD and 0.03µg/ml and 0.05µg/ml for LP respectively. The sensitivity of the method was found to be 6.6µg/cm^3/AU and 5.3µg/cm^3/AU. The results of assay showed that the amount of drug was in good agreement with the label claim of formulation as indicated by % assay (99.08%) for AMD and (100.06%) for LP.

As there is no interference of the degraded components with the standard drug absorbance at their wavelengths, hence the proposed method was specific for the estimation of Amlodipine besylate and Losartan potassium.

CONCLUSION

All the above factors lead to the conclusion that the proposed method is simple, accurate, precise, sensitive, robust and cost effective and can be applied successfully for the estimation of AMD and LP in bulk drug and marketed formulation.

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