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RESEARCH ARTICLE

SPECTROPHOTOMETRIC SIMULTANEOUS ESTIMATION OFAMLODIPINE BESYLATE AND LISINOPRIL DIHYDRATE IN TABLET DOSAGE FORMS

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ABSTRACT

Amlodipine Besylate and Lisinopril dihydrate are used for the treatment of hypertension. A binary mixture of Amlodipine Besylate (AML) and Lisinopril dihydrate (LSD) was determined by UV spectroscopic methods. The major aim of this research work is to develop simple, economical, accurate, and precise methods for simultaneous estimation of Amlodipine Besylate (AML) and Lisinopril dihydrate (LSD) in tablet dosage form. The method involved to solve simultaneous equations based on measurement of absorbance at two wavelengths 240nm (λmax of Amlodipine Besylate (AML)) and 218nm (λmax of Lisinopril dihydrate (LSD)). The two drugs followed Beers-Lamberts law over the concentration range of 2µg/ml-30µg/ml for AML and 2µg/ml -30µg/ml for LSD. The accuracy and precision of the methods were determined and the methods validated statically and this technique may be employed to analysed the drug containing Amlodipine besylate (AML) and Lisinopril dihydrate (LSD).

Key words:, Amlodipine Besylate, Lisinopril dihydrate , UV spectroscopy, Simultaneous equation method.

1. INTRODUCTION:

Amlodipine (AML) is chemically 3-ethyl 5-methyl (4*RS*)- 2-[(2-aminoethoxy) methyl]- 4- (2-chlorophenyl) -6-methyl-1,4- dihydropyridine-3,5- dicarboxylate benzene sulphonate. It is used as Antihypertensive & antianginal agent[1]. Amlodipine act by blocking voltage-sensitive calcium channels (L-type). Amlodipine slow conduction in the SA and AV nodes where action potential propagation depends on slow inward Ca^{2+} current, slowing the heart and terminating SVT by causing partial AV block. It shortens the plateau of the action potential and reduces the force of contraction. Reduced Ca^{2+} entry reduces after depolarization and thus suppresses premature ectopic beats.[2-4]

Lisinopril dihydrate (LSD) is chemically(*S*)- 1-[N2-(1-carboxy-3-phenylpropyl)-L-lysyl]-L-prolinedihydrate. It is also used as Antihypertensive agent.[5] Lisinopril is a potent, competitive inhibitor of angiotensin-converting enzyme (ACE), the enzyme responsible for the conversion of angiotensin I (ATI) to angiotensin II (ATII). ATII regulates blood pressure and is a key component of the renin-angiotensinaldosterone system (RAAS). [6-8] Lisinopril may be used to treat hypertension and symptomatic congestive heart failure, to improve survival in certain individuals following myocardial infarction and to prevent progression of renal disease in hypertensive patients with diabetes mellitus and microalbuminuria or overt nephropathy.[9-10]

2. MATERIAL AND METHODS:

Instrument:

A Shimadzu UV/Visible spectrophotometer (1700, Shimadzu,Japan) was employed with spectral bandwidth of 2nm and wavelength accuracy of ± 0.5 nm with automatic wavelength correction with a pair of 10mm quartz cells.

Chemicals:

Amlodipine besylate and Lisinopril dihydrate (were procured as a gift sample from LUPIN Pharmaceutical Ltd.,Bhopal, India). The commercial pharmaceutical formulation (Amtas-LP, Intas, Ahemdabad) tablet was procured from the local market. Methanol AR grade and Hydrochloric acid was procured from Cipla.

Preparation of standard stock solutions:

The standard stock solutions of 1mg/ml of AML and 1mg/ml of LSD were prepared.100mg of both the drugs were separately taken in 100 ml volumetric flask and dissolved in methanol : 1N HCl (1:1) solutions and then volume made up to the mark with methanol : 1N HCl (1:1). Further dilutions were made to in methanol : 1N

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HCl (1:1) to obtain concentrations 10µg/ml for AML and LSD.

Determination of Absorption Maxima:

By appropriate dilution of standard stock solutions of AML and LSD with methanol : 1N HCl (1:1), solutions containing 10µg/ml of AML and 10µg/ml of LSD were scanned separately in the range of 200- 400 nm. Wavelength of maximum absorption was determined for both the drugs. AML showed maximum absorbance at 240nm and LSD at
218nm.

Simultaneous Equation Method:

From the stock solution, working standard solution of drugs were prepared by appropriate dilution and was scanned from 400nm to 200nm**.** Two wavelengths were selected for this method i.e. 240 nm and 218nm that are absorption maximas of AML and LSD, respectively in methanol :1N HCl (1:1). Series of dilution were prepared from standard solutions of AML and LSD. The linearity was observed in the concentration range of 2µg/ml-30µg/ml for AML and 2µg/ml-30µg/ml for LSD. The absorbances were measured at the selected wavelengths and absorptivities (A1%, 1cm) for both the drugs at both wavelengths were determined. The calibration curves for AML and LSD were plotted in the concentration range of 2µg/ml-30µg/ml. The concentrations of drugs in sample solution were determined by using the following formula.

$A_1 = a_{x1}C_x + a_{y1}C_y$ .....(I)

$A_2 = a_{x2}C_x + a_{y2}C_y$ ..... (II)

$$C_x = \frac{A_1 a y_2 - A_2 a y_1}{a x_1 a y_2 - a x_2 a y_1} \quad \text{(III)}$$

$$C_y = \frac{A_1 a x_2 - A_2 a x_1}{a y_1 a x_2 - a y_2 a x_1} \quad \text{(IV)}$$

A_1 and A_2 = Absorbance of sample at λ_1 and λ_2

C_x and C_y = Concentrations of AML and LSD in sample matrix.

ax_1 and ax_2 = Absorptivities of AML at λ_1 and λ_2

ay_1 and ay_2= Absorptivities of LSD at λ_1 and λ_2

By solving the two simultaneous equations, the concentrations of AML and LP in sample solutions were obtained.[11]

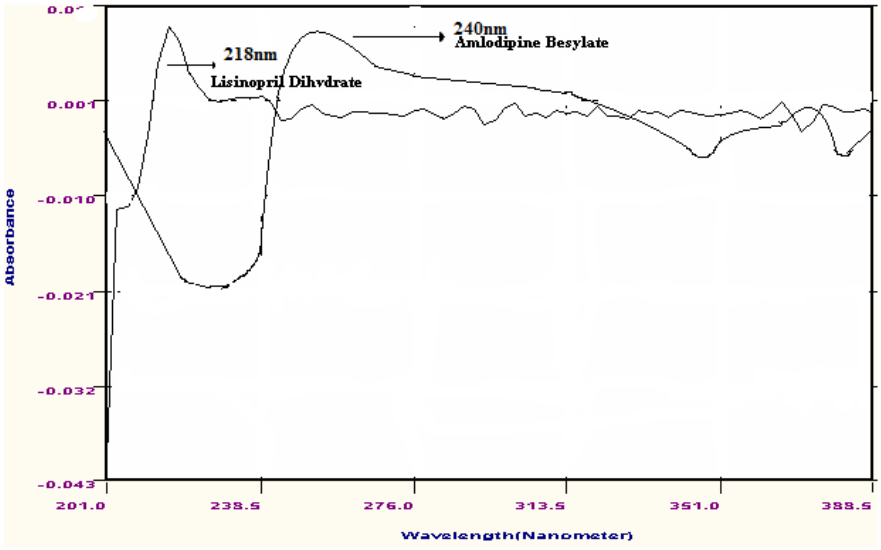


Figure 1: Scanning of overlain spectra of Amlodipine besylate & Lisinopril dihydrate in methanol : 1N HCl (1:1) solutions.

Simultaneous equation found was

At λ_{240nm} : $1.9 = 0.19C_x + 0.19C_y$ .....(1)

Atλ_{218nm} : $1.5 = 0.18C_x + 0.12C_y$ ..... (2)

Analysis of tablet formulation:

For the estimation of drugs in the commercial formulations, twenty tablets containing 5mg of AML and 5mg of LSD were weighed and average weight was calculated. The tablets were crushed and powdered in glass mortar. For the analysis of drugs, quantity of powder equivalent to 5mg of AML and 5mg of LSD was transferred to 100 ml volumetric flasks and dissolved in sufficient quantity of methanol : 1N HCl (1:1). It was sonicated for 30mins and volume was made up to obtain a stock solution of 1000µg/ml of AML and 1000µg/ml of LSD. This solution was then filtered through whatmann filter paper (grade one). Further dilutions were made from this stock solution to get required concentration.

RESULTS & DISCUSSION:

Table 1:

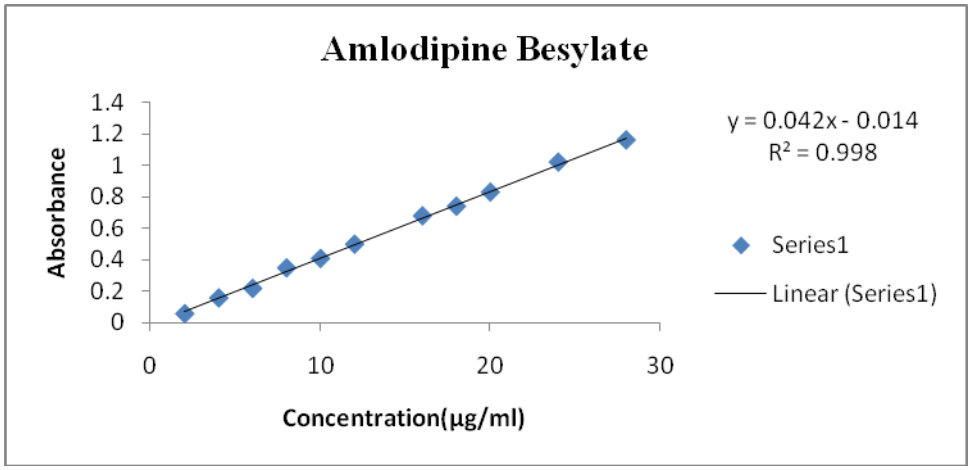
Particulars		**Result**	
Absorbance of 20 µg/ml solution of tablet at	240nm	1.154	
	218nm	0.959	
Drug		Amlodipine besylate	Lisinopril dihydrate
Drug found/ label claim (mg)		5.16*/5	5.14*/5
%found/%limit		103.2/90-110	102.8/90-110

Validation:

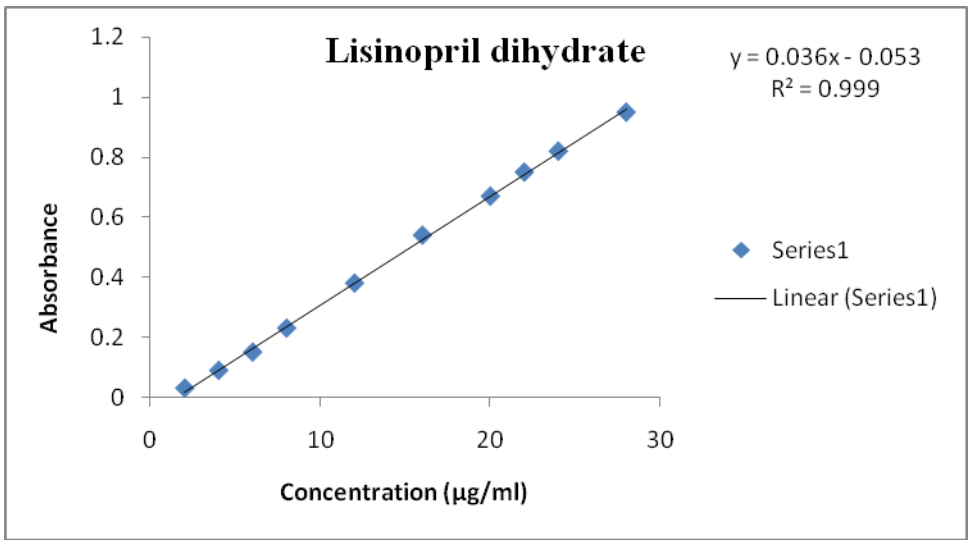
The method was validated according to ICH guidelines to study linearity, accuracy and precision.[12]

Linearity:

The linearity of measurement was evaluated by analyzing different concentrations of the standard solution of AML and LSD. The Beer law was obeyed in the concentration range 2 µg/ml -30 µg/ml for AML and LSD respectively. The correlation coefficient was found to be 0.9985 at 240 nm for Amlodipine besylate and 0.9993 at 218 nm for Lisinopril dihydrate.



Graph 1 - Calibration curve of Amlodipine Besylate



Graph 2 – Calibration curve of Lisinopril dehydrate Accuracy (Recovery studies):

To ascertain the accuracy of proposed methods, recovery studies were carried out by standard addition method at three different levels (80%, 100% and 120%). Percent recovery for AML and LSD, was found in the range of 92.3- 97.2% (Table No.2).

Table 2:

Level Recovery (%)	Drug	Conc. of Drug in µg/ml		Simultaneous Equation Method		
		Drug taken	Std drug added	% Recovery	SD	%RSD
80	AML#	50	2	92.3	0.76	0.83
100		50	2.5	93.4	1.80	1.93
120		50	3	93.6	0.90	0.97
80	LSD#	50	2	96.3	2.02	2.10
100		50	2.5	97.2	2.22	2.29
120		50	3	96.7	1.40	1.45

n=3 observations

Precision:

The reproducibility of the proposed methods was determined by performing tablet assay at different time intervals on same day (Intraday precision) and on three different days (Inter-day precision) and by three different analyst (Interanalyst).

Table 3:

Drug	**Actual conc. (µg/ml)**	**Intraday Precision**		**Interday Precision**		**Interanalyst Precision**	
		SD	**%RSD**	**SD**	**%RSD**	**SD**	**%RSD**
Amlodipine Besylate (AML)#	10	0.529	0.51	0.8	0.78	0.8	0.77
	15	0.529	0.52	0.41	0.40	0.642	0.62
	20	1.058	1.03	0.416	0.40	0.611	0.59
Lisinopril dihydrate (LSD)#	10	1.05	1.03	0.57	0.56	1.20	1.16
	15	0.50	0.49	0.61	0.59	0.94	0.91
	20	0.503	0.49	1.026	0.99	0.91	0.89

n=3 observations

RESULTS AND DISCUSSION:

The methods discussed in the present work provide a convenient and accurate way for simultaneous analysis of AML and LSD. In simultaneous equation method, wavelengths selected for analysis were 240 nm for AML and 218 nm for LSD. In this methods linearity were observed in the concentration range of 2µg/ml -30µg/ml for AML and LSD, respectively.

Accuracy of proposed method was ascertained by recovery studies and the results are expressed as % recovery. Percent recovery for AML, was found in the range of 91.5% to 95.2% & SD ± %RSD was found to be 1.241±1.33. Percent recovery for LSD, was found in the range of 94.5% to 99.6% & SD ± %RSD was found to be 1.7008±1.76. The results of validation parameters shown in table no.2 are satisfactory, indicates the accuracy of proposed methods for estimation of AML and LSD. These methods can be employed for routine analysis of the two drugs in combined tablet dosage form. The precision of the developed method was determined by preparing the tablet samples of the same batch in six determinations with three concentrations. The SD & %RSD of the assay results, expressed as a percentage of the label claim, was used to evaluate the method precision. The results are shown in Table 3, reveales the good precision of the developed method.

CONCLUSION:

The spectrophotometric methods were developed and validated as per ICH guidelines. The standard deviation and % RSD calculated for the proposed methods are within limits, indicating high degree of precision of the methods. The results of the recovery studies performed indicate the methods to be accurate. Hence, it can be concluded that the developed spectrophotometric methods are accurate, precise and can be employed successfully for the estimation of Amlodipine besylate and Lisinopril dihydrate in tablet dosage form.

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