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SIMULTANEOUS SPECTROPHOTOMETRIC ESTIMATION OF LISINOPRIL ANHYDRATE AND S-AMLODIPINE BESYLATE IN PHARMACEUTICAL DOSAGE FORM

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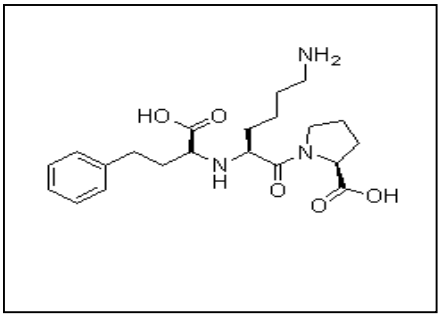
ABSTRACT

Two simple, accurate and reproducible spectrophotometric methods have been developed for the simultaneous estimation of Lisinopril anhydrate and S-Amlodipine besylate in pharmaceutical dosage forms. The first method involves determination using the Simultaneous equation method while the second method is Multicomponent mode method. For both the methods the sampling wavelengths selected were 213.5 nm and 238.5 nm over the concentration ranges of 5-30µg/mL and 5-25 µg/mL for Lisinopril anhydrate and S-Amlodipine besylate respectively. The results of the analysis were validated as per ICH guidelines and were found to be satisfactory to analyse the tablet dosage form.

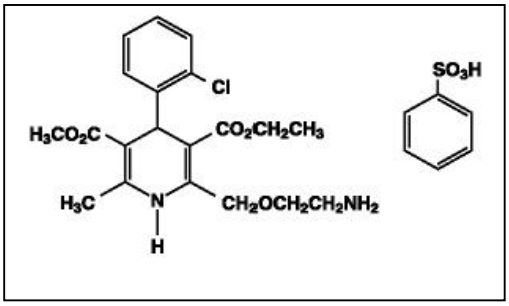
INTRODUCTION

Lisinopril anhydrate (Lisino) chemically (2S)-1-[(2S)-6-amino-2-[{(1S)-1-carboxy-3-phenylpropyl} amino] hexanoyl] pyrrole-2-carboxylic acid[1,2] is an ACE inhibitor which acts by directly blocking the formation of Angiotensin -II and at the same time increases bradykinin level. Literature survey reveals UV spectroscopic [3-4] , HPLC[5] and HPTLC[6-7] methods for the estimation of Lisino in combination with other drugs.

S-Amlodipine Besylate(S-Amlo) [8], chemically 3-Ethyl-5- methyl (4S)-2-[(2-aminoethoxy) methyl]-4-(2- chlorophenyl)-6-methyl-1, 4-dihydropyridine-3, 5- dicarboxylate benzene sulphonate is a long-acting calcium channel blocker used for hypertension and angina pectoris.[9-10]. Literature survey reveals several spectroscopic[11] , and HPTLC [12]methods for the estimation of S-Amlo in combination with other drugs.



LISINOPRIL ANHYDRATE



S-AMLODIPINE BESYLATE

Lisino and S-Amlo are available in combined pharmaceutical dosage form for the treatment of hypertension. A need was felt to develop UV methods to analyze the drugs simultaneously. This paper describes two simple, rapid, accurate, reproducible and economical methods for the simultaneous determination of Lisino and S-Amlo in tablet formulations using Simultaneous Equation Method and Multicomponent Mode Method.

EXPERIMENTAL

Instrumentation

A Shimadzu UV/Visible spectrophotometer, Model 1700 (Japan) was employed with spectral bandwidth of 2 nm and wavelength accuracy of ± 0.5 nm, with automatic wavelength correction was employed. A Denever electronic analytical balance was used for weighing the samples.

Reagents and Chemicals:

Analytical pure samples of Lisino (Piramal Healthcare Limited, Mumbai) and S-Amlo (Emcure Pharmaceutical Ltd. Pune, India) were used in the study. The pharmaceutical dosage form used in this study was Listril-SM (Torrent Pharmaceuticals Ltd., India) labelled to contain 5 mg Lisino and 2.5 mg of S-Amlo.

Preparation of Standard Stock Solution :

Standard stock solutions (100µg/mL) of Lisino and S-Amlo were prepared by dissolving separately accurately about 10 mg of drug each in 100 ml methanol. The working standard solutions of these drugs were obtained by dilution of the respective stock solution with methanol.

Preparation of Sample Stock Solutions :

Twenty tablets were accurately weighed and powdered. An accurately weighed powder sample equivalent to 5 mg of Lisino was transferred to a 100 ml volumetric flask and dissolved in methanol and sonicated for 20 minutes and volume made to 100 ml with methanol. It was then filtered through Whatmann filter paper No.42. The solution was suitably diluted with methanol to obtain sample solutions containing Lisino and S-Amlo in the concentration ratio of 2:1 µg/mL respectively as in the formulation. The final concentrations were 15 µg/mL of Lisino and 7.5 µg/mL of S-Amlo.

Construction of calibration curve

Suitable dilutions of the standards solutions were scanned in the UV range and absorbance maxima were determined. For both the methods 213.5 nm and 238.5 nm were selected as the sampling wavelengths for Lisino and S-Amlo respectively.

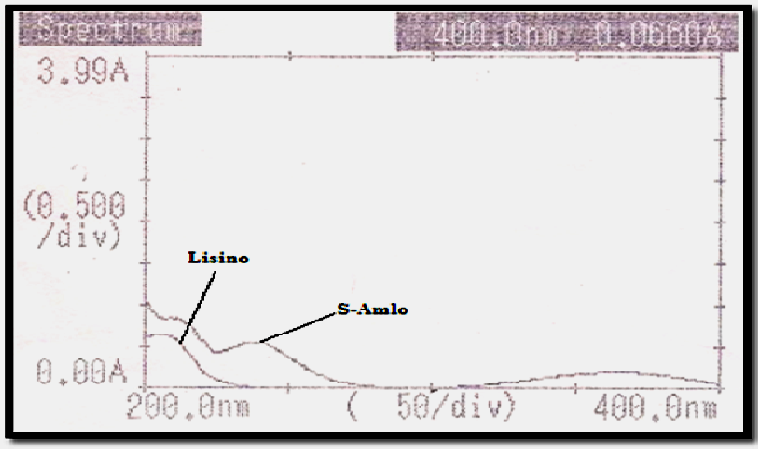


Fig.1: The overlain UV spectra of Lisino and S-Amlo.

Lisino and S-Amlo exhibited linearity with absorbances in the range of 5-30 µg/mL and 5-25 µg/mL at their respective selected wavelengths. Co-efficient of correlation was found to be 0.999 and 0.999 for Lisino and S-Amlo respectively. The optical characteristics and regression values for the calibration curves are presented in Table 1.

Method A:

Simultaneous Equation Method

For simultaneous estimation of Lisino and S-Amlo, mixed standards containing Lisino and S-amlo in a concentration ratio of 2:1 µg/mL each were prepared by appropriate dilution of the standard stock solutions with methanol. The absorbances of the mixed standard solutions were measured at the selected wavelengths. A set of two simultaneous equations were used for obtaining the concentrations of Lisino and S-Amlo as follows;

$$C_x = \frac{A_2\, ay_1 - A_1\, ay_2}{ax_2\, ay_1 - ax_1\, ay_2} \quad \text{..... Eq. (i)}$$

$$C_Y = \frac{A_1\, ax_2 - A_2\, ax_1}{ax_2\, ay_1 - ax_1\, ay_2} \quad \text{.....Eq. (ii)}$$

Where, A_1 and A_2 are absorbances of mixture at 213.5 nm and 238.5 nm respectively, ax_1 and ax_2 are absorptivities of Lisino at $\lambda_1$and λ_2 respectively and ay_1 and ay_2 are absorptivities of S-Amlo at λ1 and λ2 respectively. Cx and Cy are concentrations of Lisino and S-Amlo respectively. The concentration of Lisino and S-Amlo in mixed standard and tablet formulation can be obtained by solving equation (i) and (ii).

Method B:

Multicomponent Mode Method

For the analysis of Lisino and S-Amlo by multicomponent method of analysis, the multicomponent mode of the UV visible spectrophotometer was used. Six mixed standards in ratio of 2:1 µg/mL showing linearity within the Beer's concentration range of Lisino and S-Amlo were prepared. In multicomponent mode of the instrument, the mixed standards were scanned over the range of 190-400 nm at the selected sampling wavelengths. The overlain spectra of the six mixed standards were then employed to determine the concentration of the drugs in sample solutions. The total absorbance of a solution at a given wavelength is equal to the sum of absorbance of the individual component present. This relationship makes possible quantitative determination of the individual constituent of a mixture even if their spectrum overlaps.

Assay of Tablet Formulation:

Twenty tablets were accurately weighed and powdered. Powder equivalent to 5 mg of Lisino and 2.5mg of S-Amlo was weighed and dissolved in 100 mL methanol with the aid of ultrasonication for 20 min. The solution was then filtered through Whatmann filter paper No.42 and diluted further to obtain final concentration of 15 µg/mL of Lisino and 7.5 µg/mL of S-Amlo. The sample solutions were analyzed as per the procedure for mixed standards. The concentrations of each drug in sample solutions were calculated using equations (I) and (II) for the Simultaneous Equation Method and using the multicomponent mode of the instrument for the Multicomponent method of analysis. The results of the analysis and statistical validation data of the tablet formulation are given in Table II.

Validation:

The proposed methods were validated according to ICH Q2B guidelines for validation of analytical procedures in order to determine the linearity, sensitivity, precision and accuracy for the analyte[13].

Linearity:

The linearity of pure drugs was evaluated by analyzing different concentration of the standard solution of Lisino and S-Amlo at their respective wavelength maxima. For simultaneous equation method and multicomponent mode method, the linearity was found to be in concentration range of 5-30 µg/mL for Lisino and 5-25 µg/mL for S-Amlo.

Precision:

Precision of the methods was studied as intra-day and interday. Intra-day study was performed by analyzing, the three different concentration of drug for three times in the same day. Inter-day precision was performed by analyzing three different concentration of the drug for three days in a week. %RSD (relative standard deviation) were calculated which should be less than 2 %. The results are shown in Table III.

Accuracy:

To ascertain the accuracy of the proposed methods, recovery studies were carried out by standard addition method at three different levels (80%, 100% and 120%). The statistical validation data of recovery study are given in Table IV

Table I: Optical Characteristics And Validation Data Of Lisino And S-Amlo for method A and B

Parameters	Lisino	S-Amlo
Absorbance Maxima	213.5 nm	238.5 nm
Linearity range (µg/ml)	5-30	5-25
*Slope ± S.D.	0.028 ± 0.0021	0.034 ± 0.0032
*Intercept ± S.D.	0.0006 ± 0.0001	0.0013 ± 0.0014
*Regression coefficient (r^2) ± S.D.	0.999 ± 0.0055	0.999 ± 0.0042
*LOD (µg/ml)	0.1532	0.0579
*LOQ (µg/ml)	0.4498	0.1754

*Denotes average of six estimations.

Method A – Simultaneous Equation Method

Method B – Multicomponent mode analysis method.

Table II: Statistical Validation Data of Tablet Formulation (LISTRIL-SM).

Component	Method	Label claim (mg)	Amount found*(mg)	% Amount Found*	± S.D.*	%R.S.D.
Lisino	A	5 mg	5.003	100.07	0.0338	0.676
	B	5mg	5.001	100.02	0.0316	0.633
S-Amlo	A	2.5 mg	2.501	100.01	0.0085	0.338
	B	2.5 mg	2.49	99.85	0.0074	0.296

*Denotes average of six estimations.

Tablet Formulation, Listril-SM, manufactured by Torrent Pharmaceuticals Ltd., India.

Table-III Results for Intra-Day & Inter-Day Precision studies.

Method	Drug	Intra -Day*			Inter- Day*		
		% Mean	S.D.*	% R.S.D.*	% Mean	S.D.*	% R.S.D.*
A	Lisino	99.90	0.027	0.186	99.77	0.053	0.356
	S-Amlo	99.97	0.013	0.158	99.57	0.024	0.323
B	Lisino	99.90	0.030	0.201	99.83	0.013	0.231
	S-Amlo	99.93	0.015	0.201	99.67	0.0344	0.183

* Denotes average of six determination

Table IV: Statistical Validation Of Recovery Studies:

Level of % Recovery	Method	% Recovery*		% R.S.D.*	
		Lisino	S-Amlo	Lisino	S-Amlo
80	A	100.1	100.02	0.077	0.153
	B	99.95	99.99	0.3682	0.1334
100	A	100.4	99.98	0.743	0.262
	B	100.05	100.07	0.1765	0.1416
120	A	100.2	99.97	0.101	0.231
	B	100.07	99.99	0.1863	0.1359

*Denotes average of three estimations at each level of recovery.

RESULTS AND DISCUSSION

Under the experimental conditions described, linearity range was studied, methods were applied to analyse laboratory mixture and assay of tablet formulation were performed. The developed methods were validated as per ICH guidelines for linearity, repeatability, intermediate precision (inter-day and intra-day precision studies), accuracy, LOD and LOQ. The mean % content of tablet by method A were found to be 100.06% and 100.04% & for method B,100.02% & 99.66% for Lisino & S-Amlo respectively (Table II). The mean % recoveries of were found to be in the ranges of 99.95%-100.4% and 99.97%-100.07% for Lisino & S-Amlo respectively by both methods (Table IV).

CONCLUSION

Lisino and S-Amlo are available in combined pharmaceutical dosage form for the treatment of hypertension. Here, two simple UV spectrophotometric methods Simultaneous Equation Method, Multicomponent Mode Method were developed for their simultaneous analysis. The standard deviation, relative standard deviation (RSD) calculated for the methods are low, indicating high degree of precision of the methods. The RSD is also less than 2% as per ICH guidelines. The developed methods are simple, rapid, precise, accurate and can be employed for the routine estimation of Lisino and S-Amlo in both bulk and tablet dosage form. Method B has advantages over the Method A, as it does not involve any manual calculations. It directly gives the concentration of the components in the sample mixture. The method using multicomponent mode in a spectrophotometer is excellent for rapid analysis in a simple and convenient way.

List of symbols and Abbreviations:

1. % : Percent
2. nm : Nanometer
3. µg/mL : Microgram Per Millilitre
4. UV : Ultraviolet
5. HPLC : High Performance Liquid Chromatography
6. HPTLC : High Performance Thin Layer Chromatography
7. S-Amlo : S- Amlodipine Besylate
8. Lisino : Lisinopril Anhydrate
9. ICH : International Conference on Harmonization
10. SD : Standard Deviation
11. RSD : Relative Standard Deviation
12. LOD : Limit of Detection
13. LOQ : Limit of Quantitation

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