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Simultaneous UV Spectrophotometric Methods for Estimation of Losartan Potassium and Amlodipine Besylate in Tablet Dosage Form

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ABSTRACT

Two simple and sensitive methods have been developed for simultaneous estimation of losartan potassium (LSP) and amlodipine besylate (AMB) in tablet dosage form which require no prior separation. Method I is simultaneous equation and Method II is first order derivative method. In simultaneous equation method 258 nm and 367 nm analytical wavelengths were selected for estimation of both drugs in glacial acetic acid as a solvent. For first order derivative method 295 nm and 326 nm were selected as analytical wavelengths for estimation of both the drugs. Recovery studies were found within a range of 99.68 % - 100.12 % for LSP and 99.52 % - 99.61 % for AMB. The proposed methods are recommended for routine analysis of LSP and AMB in pharmaceutical dosage form where no heating and organic solvent extraction is required.

Keywords: Losartan potassium, Amlodipine besylate, Simultaneous equation method, First order derivative method.

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INTRODUCTION

Losartan (LSP) chemically, 1,2-n-butyl-4-chloro-1-[p-(o-1H-tetrazol-5-ylphenyl)benzyl]imidazole-5-methanol monopotassium salt is a highly selective, orally active, non-peptide angiotensin II receptor antagonist indicated for the treatment of hypertension. It has a more potent active metabolite EXP3174 (II, 2-n-butyl-4-chloro-1-[2-(1H-tetrazol-5 yl) biphenyl- 4-yl] methyl] imidazole-5-carboxyl acid) [1]. The determination of LSP has been carried out in tablets by HPLC, capillary electrophoresis and supercritical fluid chromatography [2-3], gas chromatography-mass spectrometer [4] and simultaneously with its active metabolite in biological fluids, by HPLC [6-8].

Amlodipine besylate (AMB), chemically, 2-[(2- aminoethoxy) methyl] - 4- (2-chlorophenyl) -1, 4-dihydro- 6-methyl-3, 5- pyridinedicarboxylic acid 3-ethyl, 5-methyl ester, is an antihypertensive and an antianginal agent in the form of the besylate salt. It is official in Indian Pharmacopoeia [4]. Various analytical methods have been reported for the assay of AMB in pure form as well as in pharmaceutical formulations. These include high performance liquid chromatography [12-13], reversed phase high performance liquid chromatography [3-4], high performance thin layer chromatography [12,13], gas chromatography [14], gas chromatography-mass spectrometry [15], liquid chromatography with tandem mass spectrometry [16], fluorimetry [15], derivative spectroscopy [17,18], simultaneous multicomponent mode of analysis and difference spectrophotometry [20,21].

The objective of the present work is to develop and validate a new UV spectrophotometric method for simultaneous determination of LSP and AMB in tablet dosage form by simultaneous equation and first order derivative method.

MATERIALS AND METHODS

Materials

Spectral runs were made on a Jasco-V630 UV-Visible spectrophotometer (Japan) with spectral bandwidth of 0.5 nm and wavelength accuracy of ± 0.3 nm with automatic wavelength corrections with a pair of 10 mm quartz cells. LSP was provided by Lupin Research Park, Pune while AML reference standard was kindly provided by Shreya Life Sciences Pvt. Ltd. Aurangabad (M.S.). The pharmaceutical formulation of combined dosage form is procured from local market. Glacial acetic acid was purchased from Loba Chemie Pvt. Ltd. (India).

Selection of common solvent

Glacial acetic acid of analytical reagent grade was selected as a common solvent for developing spectral characteristics of both drugs. The selection was made after assessing the solubility of both the drugs in different solvents.

Preparation of standard stock solution

Standard stock solutions (100 $\mu\text{g}/\text{ml}$) of LSP and AMB were prepared by dissolving separately 10 mg of each drug in 20 ml of glacial acetic acid and volume was made up to 100 ml with distilled water. The working standard solutions of these drugs were obtained by dilution of the respective stock solution with distilled water.

Analysis of pharmaceutical dosage form

Twenty tablets were weighed and crushed to a fine powder. An accurately weighed powder sample equivalent to 50 mg of LSP was transferred to a 100 ml volumetric flask and dissolved in 20 ml of glacial acetic acid. After the immediate dissolution, the volume was made up to the mark with the distilled water and was then filtered through Whatmann filter paper No.41. The solution was suitably diluted with distilled water to obtain sample solutions containing LSP and AMB in the concentration ratio of 1:10 $\mu\text{g}/\text{ml}$ respectively as in the tablet formulation.

Simultaneous equation method

For the simultaneous equation method, 258 nm and 367 nm were selected as the two sampling wavelengths for LSP and AMB respectively. The Fig.1 represents the overlain UV spectra of LSP and AMB. The LSP and AMB exhibited linearity in the concentration range of 20-100 $\mu\text{g}/\text{ml}$ and 10-60 $\mu\text{g}/\text{ml}$ at their respective selected wavelengths respectively. Coefficients of correlation were found to be 0.998 and 0.999 for LSP and AMB respectively. The optical characteristics and regression values for the calibration curves are presented in Table 1. For simultaneous estimation of LSP and AMB, mixed standards containing LSP and AMB in a concentration ratio of 1:10 $\mu\text{g}/\text{ml}$ each were prepared by appropriate dilution of the standard stock solutions with distilled water. The absorbances of the mixed standard solutions were measured at the selected wave lengths.

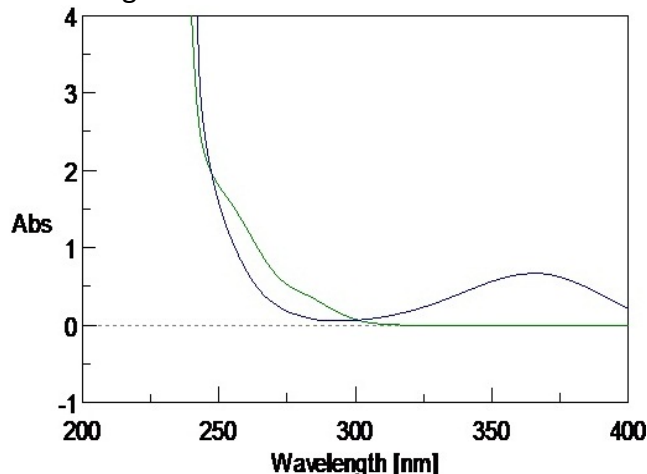


Fig 1: Overlain spectra of LSP and AMB for simultaneous equation method

The two equations were constructed based upon the fact that at λ_1 and λ_2 the absorbance of the mixture is the sum of individual absorbances of LSP and AMB.

$$\text{At } \lambda_1, A_1 = ax_1bc_x + ay_1bc_y \dots\dots (1)$$

$$\text{At } \lambda_2, A_2 = ax_2bc_x + ay_2bc_y \dots\dots (2)$$

Where, A_1 and A_2 are absorbances of mixed standard at 258 nm and 367 nm respectively. λ_1 and λ_2 are wavelengths of LSP and AMB respectively, ax_1 and ax_2 are absorptivity of LSP at λ_1 and λ_2 , ay_1 and ay_2 are absorptivity of AMB at λ_1 and λ_2 respectively. c_x and c_y are concentration of LSP and AMB respectively.

First order derivative method

LSP and AMB were scanned separately in a wavelength range of 200-400 nm against distilled water blank. The first order derivative spectra were obtained by instrumental electronic differentiation in the range of 200 to 400 nm. A signal at 326 nm of first derivative spectrum was selected for quantification of AMB where no interference due to LSP was observed similarly a signal at 295 nm was selected for quantification of LSP, where AMB did not interfere with the estimation of LSP. A first order derivative overlain spectrum of LSP and AMB is shown in Fig. 2

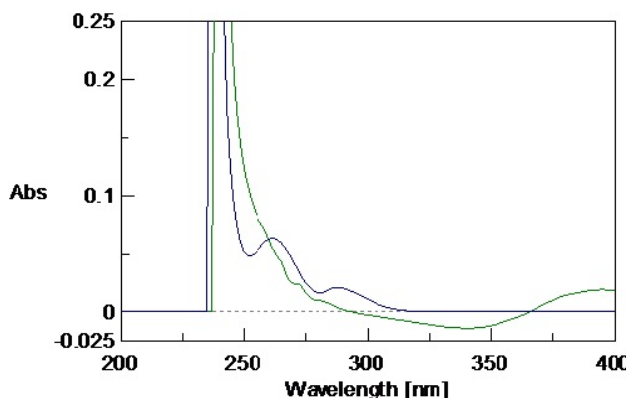


Fig 2: Overlain spectra of LSP and AMB for first order derivative

Validation

The method was validated according to ICH guidelines for validation of proposed analytical method in order to determine the linearity, sensitivity, precision and accuracy for the analyte.

Accuracy

To ascertain the accuracy of the proposed method, recovery studies were carried out by standard addition method at three different levels (80%, 100% and 120%). Percent recovery for LSP and AMB by the proposed method was found in the range of 99-101%.

Linearity

The linearity of measurement was evaluated by analyzing different concentrations of the standard solution of LSP and AMB. For simultaneous equation method the Beer- Lambert's concentration range was found to be 10-60 $\mu\text{g/ml}$ for LSP 20-100 $\mu\text{g/ml}$ for AMB.

Precision

Precision was studied to find out intra and inter-day variations in the test method of LSP and AMB. Calibration curves prepared were run in triplicate in same day and for three days. Relative standard deviation (% RSD) was calculated which should be less than 2 %.

RESULTS AND DISCUSSION

Under the experimental conditions described, calibration curve, assay of tablets and recovery studies were performed. The developed methods were validated as per ICH guidelines for linearity, repeatability, LOD, LOQ as shown in Table 1. Obedience to Beer's law was confirmed by linearity of the calibration curve of LSP and AMB, which are representing in Fig.3 and 4. The mean % content of LSP and AMB in tablet formulation by the simultaneous equation method was 99.68% and 99.61% respectively and by first order derivative method was 99.33% and 99.52% respectively (Table 2). The mean % recoveries of LSP and AMB were found to be 99.82 % and 98.58 % respectively by simultaneous equation method and 99.74% and 100.04% respectively by first order derivative method (Table 3).

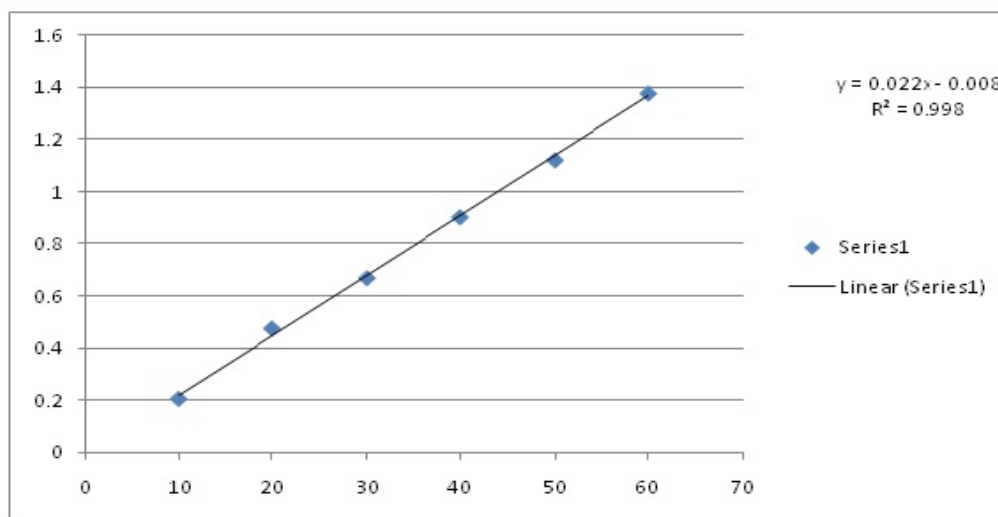


Fig 3: Calibration curve for LSP for simultaneous equation method

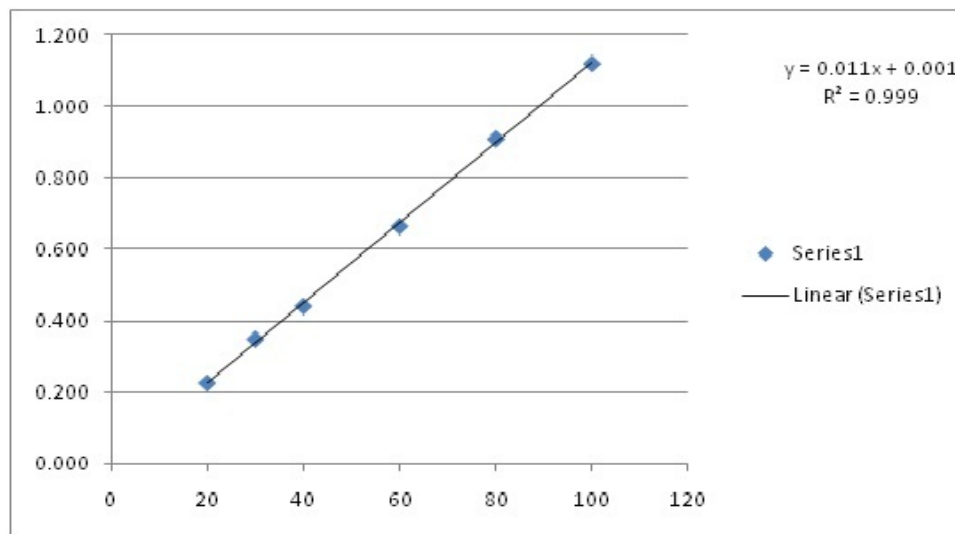


Fig 4: Calibration curve for AMB for simultaneous equation method

Table 1: Optical Characteristics and Validation Parameters of LSP and AMB

Parameter	LSP		AMB	
	Method I	Method II	Method I	Method II
λ_{max}	258 nm	295 nm	367 nm	326 nm
Beer's Law range ($\mu\text{g/ml}$)	10-60	10-60	20-100	20-100
Precision (%RSD)	1.31	0.976	0.645	1.012
LOD ($\mu\text{g/ml}$)	0.3248	0.345	1.153	0.536
LOQ ($\mu\text{g/ml}$)	0.984	0.931	3.496	0.956
Regression values: Y = mx + C				
I Slope	0.017	0.0003	0.058	0.0002
II Intercept	0.008	0.0002	0.001	0.0002
III Regression coefficient(r^2)	0.998	0.9995	0.999	0.9996

Table 2: Analysis of Pharmaceutical Dosage Form

Drug	Method	Label Claim (mg/tab)	% Amount Found	S.D.*	% R.S.D
LSP	I	50	99.68	0.1727	0.645
	II		99.33	0.854	0.8519
AMB	I	5	99.61	0.1659	1.31
	II		99.52	0.887	0.8764

*S.D. = Standard Deviation, Mean of six replicates

Table 3: Statistical Validation of Recovery Studies

Level of recovery (%)	Method	%Recovery**		% R.S.D	
		LSP	AMB	LSP	AMB
80	I	99.68	99.61	0.645	1.31
	II	98.98	99.92	0.358	0.931
100	I	99.68	99.61	0.645	1.31
	II	99.94	99.96	0.375	0.912
120	I	100.12	99.52	0.612	1.25
	II	100.20	100.24	0.380	0.876

**Mean of three replicates

CONCLUSION

Two simple, precise, accurate and sensitive spectrophotometric methods has been developed for simultaneous estimation of LSP and AMB in combined tablet dosage form by simultaneous equation method and by first order derivative method. The values for standard deviation and RSD are found to be low, indicating high degree of precision of the method. The % recovery was found between 99-101% indicating good accuracy of the proposed methods. The proposed methods can be employed for the routine estimation of LSP and AMB in both bulk and tablet dosage form.

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