ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES Knowledge to Innovation

Vol 7, Issue 4, 2014

ISSN - 0974-2441

Research Article

SPECTROPHOTOMETRIC SIMULTANEOUS ESTIMATION OF AMLODIPINE BESYLATE AND LOSARTAN POTASSIUM IN TABLET DOSAGE FORMS

MANISHA MASIH[1], ABHILASHA MITTAL[2], NANDY BC[2]

[1]PhD. Research Scholar, Department of Pharmaceutical Science, [2]Jayoti Vidyapeeth Womens University, Vedant Gyan Valley, Jharna Mahala Jobner Link Road, NH-8, Jaipur Rajasthan-303 122, India. Email: manishamasih85@gmail.com

Received: 13 June 2014, Revised and Accepted: 11 July 2014

ABSTRACT

Amlodipine (AML) besylate and losartan potassium (LSP) are used for the treatment of hypertension. A binary mixture of AML besylate and LSP was determined by UV spectroscopic methods. The major aim of this research work was to develop simple, economical, accurate, and precise methods for simultaneous estimation of AML and LSP in tablet dosage form. The method involved to solve simultaneous equations based on measurement of absorbance at two wavelengths 240 nm (λ max of AML) and 220 nm (λ max of LSP). The two drugs followed beers-lamberts law over the concentration range of 2 µg/ml-30 µg/ml for both the drugs (AML and LSP). The accuracy and precision of the methods were determined, and the methods validated statically and this technique may be employed to analyze the drug containing AML besylate and LSP.

Keywords: Amlodipine besylate, Losartan potassium, UV spectroscopy, Simultaneous equation method.

INTRODUCTION

Amlodipine (AML) is chemically 3-ethyl5-methyl (4RS)-2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5- dicarboxylate benzene sulphonate. It is used as antihypertensive and antianginal agent [1]. AML act by blocking voltage-sensitive calcium channels (L-type). AML causes slow conduction in the sinoatrial and atrioventricular nodes, where action potential propagation depends on slow inward Ca^{2+} current, slowing the heart and terminating supraventricular tachycardia by causing partial atrioventricular (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].

Losartan potassium (LSP) is chemically 4-butyl-4-chloro-1-[[2′-(1*H* tetrazol-5-yl) [1,1′-biphenyl]-4-yl]methyl]-1*H*-imidazole-5-methanol. It is also used as antihypertensive agent [5]. Losartan is a competitive antagonist and inverse antagonist of the A-II receptor. It blocks the actions of A-II, *viz.* Vasoconstriction, release of aldosterone and adrenaline from adrenals, renal actions promoting salt and water reabsorption, and central actions such as thirst, vasopressin release, and growth promoting actions on heart and blood vessels [6-10].

MATERIALS AND METHODS

Instrument

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

Chemicals

AML besylate and LSP were procured as a gift sample from LUPIN Pharmaceutical Ltd., Bhopal, India. The commercial pharmaceutical formulation (Amlopress-Z, Cipla, Sikkim, India) tablet was procured from the local market. Methanol AR grade and hydrochloric acid were procured from Cipla, Indore, Madhya Pradesh, India.

Preparation of standard stock solutions

The standard stock solutions of 1 mg/ml of AML and 1 mg/ml of LSP were prepared. 100 mg of both the drugs was separately taken in 100 ml volumetric flask and dissolved in methanol:1 N HCl (1:1) solutions and then volume made up to the mark with methanol:1 N HCl (1:1). Further dilutions were made with the methanol:1 N HCl (1:1) solutions to obtain concentrations 10 µg/ml of AML and LSP.

Determination of absorption maxima

By appropriate dilution of standard stock solutions of AML and LSP with methanol:1 N HCl (1:1), solutions containing 10 µg/ml of AML and 10 µg/ml of LSP 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 240 nm and LSP at 220 nm.

Simultaneous equation method

From the stock solution, working standard solutions of drugs were prepared by appropriate dilution and were scanned from 400 to 200 nm. Two wavelengths were selected for this method, i.e. 240 nm and 220 nm that are absorption maxima of AML and LSP, respectively, in methanol:1 N HCl (1:1). Series of dilution were prepared from standard solutions of AML and LSP. The linearity was observed in the concentration range of 2 µg/ml-30 µg/ml for AML and 2 µg/ml-30 µg/ml for LSP. The absorbance was measured at the selected wavelengths and absorptivities (A1%, 1 cm) for both the drugs at both wavelengths were determined. The calibration curves for AML and LSP were plotted in the concentration range of 2 µg/ml-30 µg/ml. The concentrations of drugs in the sample solution were determined by using the following formula.

$$A_1 = a_{x1}C_x + a_{y1}C_y \quad \text{(I)}$$

$$A_2 = a_{x2}C_x + a_{y2}C_y \quad \text{(II)}$$

$$C_x = \frac{A_1 ay_2 - A_2 ay_1}{ax_1 ay_2 - ax_2 ay_1} \quad \text{(III)}$$

$$C_y = \frac{A_1 ax_2 - A_2 ax_1}{ay_1 ax_2 - ay_2 ax_1} \quad \text{(IV)}$$

A_1 and A_2 = Absorbance of the sample at λ_1 and λ_2

C_x and C_y = Concentrations of AML and LSP in the sample matrix

ax_1 and ax_2 = Absorptivities of AML at λ_1 and λ_2

ay_1 and ay_2= Absorptivities of LSP at λ_1 and λ_2

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

Analysis of tablet formulation

For the estimation of drugs in the commercial formulations, 20 tablets containing 5 mg of AML and 50 mg of LSP were weighed, and average weight was calculated. The tablets were crushed and powdered in a glass mortar. For the analysis of drugs, quantity of powder equivalent to 5 mg of AML and 50 mg of LSP was transferred to 100 ml volumetric flasks and dissolved in sufficient quantity of methanol:1 N HCl (1:1). It was sonicated for 30 minutes, and volume was made up to obtain a stock solution of 1000 µg/ml of AML and 1000 µg/ml of LSP. This solution was then filtered through whatman filter paper (grade one). Further dilutions were made from this stock solution to get required concentration.

RESULTS AND DISCUSSION

Simultaneous equation found was

$$\text{At } \lambda_{240nm}: 1.248 = 0.146C_x + 0.166C_y \quad (1)$$

$$\text{At } \lambda_{220nm}: 0.984 = 0.12C_x + 0.126C_y \quad (2)$$

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 LSP. The beer law was obeyed in the concentration range 2 µg/ml-30 µg/ml for AML and LSP, respectively. The correlation coefficient was found to be 0.9985 at 240 nm for AML besylate and 0.9986 at 220 nm for LSP.

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 LSP, was found in the range of 92.3-98.1% (Table 2).

Precision

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

The methods discussed in the present work provided a convenient and accurate way for simultaneous analysis of AML and LSP. In simultaneous equation method, wavelengths selected for analysis were 240 nm for AML and 220 nm for LSP. In these methods, linearity was observed in the concentration range of 2 µg/ml-30 µg/ml for AML and LSP, respectively.

Accuracy of the proposed method was ascertained by recovery studies, and the results are expressed as % recovery. Percent recovery for AML, was found in the range of 91.5-95.2% and standard deviation (SD)± % relative standard deviation (RSD) was found to be 1.241±1.33. Percent recovery for LSP, was found in the range of 94.5-99% and SD ± %RSD was found to be 1.564±1.61. The results of validation parameters shown in Table 2 which are satisfactory, and it indicated the accuracy of proposed methods for estimation of AML and LSP. 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 and %RSD of the assay results, expressed as a percentage of the label claim, were used to evaluate the method precision. The results are shown in Table 3, revealed 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 a 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 AML besylate and LSP in tablet dosage form.

Table 1: Analysis of tablet of AML and LSP through UV-visible spectroscopic method

Particulars	Result	
Equivalent to 20 µg/ml of AML and LSP of tablets triturates		
240 nm	1.051	
220 nm	0.895	
Drug	**AML besylate**	**LSP**
Drug found/label claim (mg)	5.19 mg*/5 mg	5.13 mg*/5 mg
%found/%limit	103.8/90-110%	102.6/90-110%

AML: Amlodipine, LSP: Losartan potassium.

Table 2: Recovery studies of AML and LSP

Drug	Level recovery	Concentration of drug in µg/ml		Simultaneous equation method		
		Drug taken	Standard drug added	% Recovery	SD	% RSD
AML	80	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
LSP	80	50	2	95.8	1.25	1.31
	100	50	2.5	98.1	1.22	1.25
	120	50	3	97.8	1.40	1.43

AML: Amlodipine, LSP: Losartan potassium, SD: Standard deviation, RSD: Relative standard deviation

Table 3: Inter day, intra-day, and inter analyst precision studies of AML and LSP

Drug	Actual concentration (µg/ml)	Intra-day precision		Inter-day precision		Inter-analyst precision	
		SD	% RSD	SD	% RSD	SD	% RSD
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
LSP#	10	0.452	0.44	0.723	0.71	0.912	0.90
	15	1.150	1.13	0.337	0.33	0.84	0.83
	20	0.306	0.30	0.733	0.72	0.491	0.49

AML: Amlodipine, LSP: Losartan potassium, #*n*=3 observations, SD: Standard deviation, RSD: Relative standard deviation

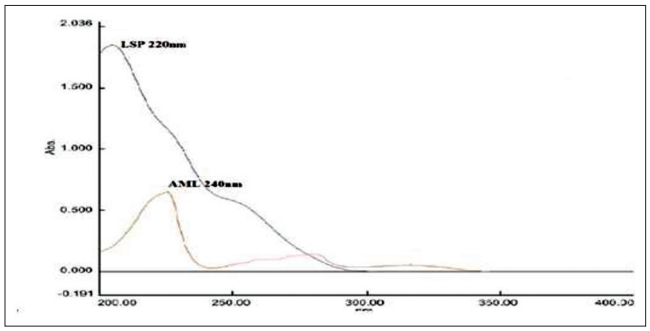
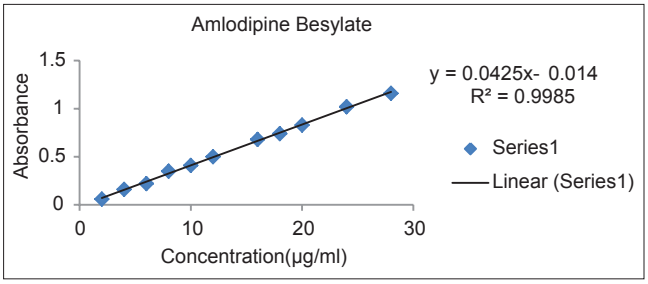
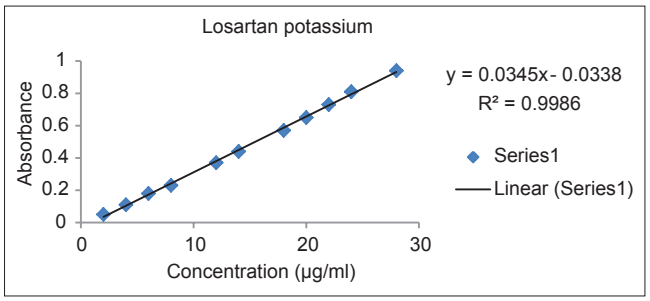


Fig. 1: Scanning of amlodipine besylate and losartan potassium in methanol:1 N HCl (1:1) solutions



Graph 1: Calibration curve of amlodipine besylate



Graph 2: Calibration curve of losartan potassium

ACKNOWLEDGMENT

The authors express their gratitude to LUPIN Pharmaceutical Ltd., Bhopal, India for Gift samples of pure drugs.

REFERENCES

1. Indian Pharmacopoeia. Vol. II. New Delhi: Published by the Controller of Publication; 2014. p. 1045-7.
2. Block JH, Beale JM, editors. Wilson and Grisvold's Textbook Organic Medicinal and Pharmaceutical Chemistry. London, UK: Wolters Kluwer; 2004. p. 631.
3. Tripathi KD. Essentials of Medical Pharmacology. 5th ed. New Delhi: Jaypee Brothers Medical Publishers; 2004. p. 496.
4. Garg G, Saraf S, Saraf S. Development and validation of simultaneous estimation of enalapril maleate and amlodione besylate in combined dosage forms. Trends Appl Sci Res 2008;3(3):278-84.
5. Indian Pharmacopoeia. Vol. II. New Delhi: Published by the Controller of Publication; 2014. p. 2122-4.
6. Block JH, Beale JM, editors. Wilson and Grisvold's Textbook Organic Medicinal and Pharmaceutical Chemistry. London, UK: Wolters Kluwer; 2004. p. 648.
7. Tripathi KD. Essentials of Medical Pharmacology. 5th ed. New Delhi: Jaypee Brothers Medical Publishers; 2004. p. 453-4.
8. Saravanan C, Srinivasan S, Narayanaswamy VB, Suresh S, Sivakuma T. Analytical method development and validation of amlodipine besylate and losartan potassium in tablet dosage form by RP-HPLC method. Int J Pharm Sci 2010;2(3):822-6.
9. Patil PR, Rakesh SU, Dhabale PN, Burade KB. Simultaneous UV spectrophotometric method for estimation of losartan potassium and amlodipine besylate in tablet dosage form. Asian J Res Chem 2009;2(1):183-7.
10. Patil PR, Rakesh SU, Dhabale PN, Burade KB. RP-HPLC method for simultaneous estimation of Losartan potassium and amlodipine besylate in tablet formulation. Int J ChemTech Res 2009;1(3):464-9.
11. Beckett AH, Stenlake JB. Practical Pharmaceutical Chemistry. 4th ed., Part 2. New Delhi: CBS Publishers & Distributors; 2004. p. 286-7.
12. ICH Harmonised Tripartite Guidelines. Validation of Analytical Procedures: Text & Methodology, Q2 (R); Nov, 2005.