RESEARCH ARTICLE

Development and Validation of RP-HPLC Method using Photodiode Array or Diode-Array Detection Detector for simultaneous Estimation of the Amlodipine Besylate and Lisinopril in Fixed-Dose Formulation

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ABSTRACT

A basic and specific HPLC-PDA (high-performance liquid chromatography-photodiode array) detector showing approach was created for the simultaneous estimation of anti-hypertensive medications—amlodipine besylate (AMD) and lisinopril (LIS). A successful chromatography method was developed using the SunFire C8 column (4.6 × 150 mm, 5 µm) with gradient elution of the mobile phase composed of 85:25 potassium dihydrogen phosphate in water and methanol at a flow rate of 0.7 mL/min. The wavelength was set at 212 nm for the simultaneous estimation of AMD and LIS. The retention time obtained was 5.1 and 10.5 minutes for AMD and LIS, respectively. Descriptive performance of the proposed HPLC methodology was measurably approved as for framework accuracy, linearity, robustness, specificity, precision, and forced degradation for identification and evaluation limits. The linearity range for AMD and LIS correlation coefficient value obtained was $R^2 > 0.999$. The drugs were exposed to forced degradation conditions—acidic, alkali, oxidation, etc. The newly developed reverse phase high performance liquid chromatography (RP-HPLC) method was applied for the detection of the referred to anti-hypertensive medications in their combination pharmaceutical tablets.

Keywords: Amlodipine besylate, Anti-hypertensives, High-performance liquid chromatography, Lisinopril, Photodiode array (PDA).

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INTRODUCTION

AMD is chemically 3-ethyl 5-methyl 2-[(2-aminoethoxy) methyl]-4-(2chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate benzene sulfonate (Figure 1).[1] AMD belongs to the third generation calcium antagonists, which retain tissue selectivity and show favorable pharmacokinetic data. AMD delivers a well good therapeutic possibility for the treatment of hypertension, and potential agents for patients with angina pectoris.[2] The AMD possess its affinity to L-type calcium channels, and further, blocking the movement of calcium ions in the heart and smooth muscles of the coronary and peripheral vascular. This response of AMD improves blood pressure in angina and recovers the bloodstream to the myocardium.[3]

LIS is also an anti-hypertensive drug, which inhibits angiotensin-converting enzyme (ACE) competitively. Chemically, it is N2-[(1S)-1-carboxy-3-phenylpropyl]-L-lysyl-L-proline (Figure 1). The major therapeutic indications

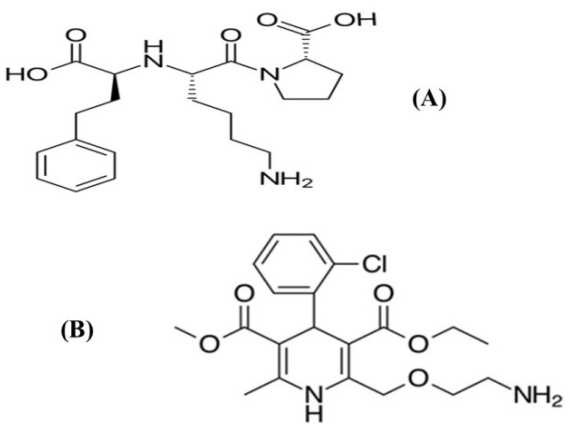

Figure 1: Chemical structure: **(A)** AMD; **(B)** LIS

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are congestive heart failure, and heart attacks, and similarly in inhibiting renal and retinal problems of diabetes.[4] The literature exposed several spectrophotometric methods, such as, high-performance thin layered chromatography (HPTLC), UV-visible spectroscopy, and high-performance liquid chromatography (HPLC) for estimation of AMD individually or in combination. Similarly, various methods, such as, HPTLC[5] and HPLC[6] methods have been discussed in previous reports for the estimation of LIS individually or in combination.

Literature is also in agreement that UV-vis spectrophotometric methods could be used for quantification estimation of AMD and LIS in a fixed combination form.[7] Consequently, HPLC is the widely used method of selecting for the immediate resolution of these two drugs. The structural configuration of LIS sorts it a complex analyst because it occurs as an asymmetrical mixture of cis- and trans-conformers due to the isomerization.[7] It has been confirmed by HPLC that chromatographic conditions, such as, flow rate, temperature, pH, and the organic transformer has an important effect on the peak shape and the retention time of LIS.[8] Available methods of drug estimation have constrained that they have high UV cut-off standards affecting baseline interferences and also, not the good alternates when low UV wavelengths for detection and quantification are used. Similarly, the estimation of AMD by the HPLC method is not so difficult and hard used alone, but it becomes difficult to quantify when combined with LIS. There are also numerous developed HPLC methods for the quantification of LIS in combination with AMD or with other active ingredients.[7] Therefore, in the current study, the authors have developed the RP-HPLC method of AMD and LIS fixed combined dosage form and validated using the International Conference on Harmonization (ICH) guidelines.[9]

METHODS

Chemicals and Solvents

HPLC grade water, methanol, potassium dihydrogen phosphate, and triethylamine were purchased from Sigma Aldrich India Pvt. Ltd. AMD reference standard 99.5% and LIS reference standard 90.5% potency were taken from Reference Division, Indian Pharmacopeia Commission (IPC), Ghaziabad, UP. Double distilled water and test tablets have been prepared at lab-scale only.

Equipment and Chromatographic Conditions

The analysis was performed with a Thermo Scientific HPLC system with a PDA detector for method development studies of AMD and LIS. Obtained HPLC data was processed using inbuilt software (Chromeleon 7.2 SR4). Hydrolysis of samples was done in a water bath (make: Bio-Gene Ltd.), and thermal stability studies in the oven. The SunFire C8 5 µm column (4.6 × 150 mm) was used as a stationary phase. HPLC chromatographic system details are provided in Table 1. Weighing of all materials was done on Shimadzu balance (Model AY-120).

Preparation of Stock and Working Solutions

Preparation of Mobile Phase

The mobile phase was prepared in a proportion of 85:15 of buffer solution and methanol, respectively. The buffer solution was prepared by dissolving the required quantity (2.72 grams) of potassium dihydrogen phosphate in 1,000 mL of water. The pH of the solution was adjusted to 7, by adding trimethylamine (TEA) in the mobile phase.

Preparation of Standard Stock Solutions

Standard stock solutions of AMD and LIS were prepared in two steps. Firstly, individual stock solutions were prepared by dissolving accurately weighed quantity of AMD (35 mg) and LIS (25 mg) in 50 mL of the mobile phase, using a volumetric flask separately, and eventually, 5 mL solution of AMD and LIS were taken together in another volumetric flask, and finally diluted to 50 mL to get 50 ppm concentration of each drug solution.

Preparation of Sample Stock

Sample solutions of marketed tablet formulation were also prepared. For this, an accurately weighed portion of the powder containing 5 mg of AMD and LIS was dissolved in 50 mL of mobile phase in a volumetric flask and sonicated for 15 minutes, 10 mL of this solution was taken and diluted to 20 mL with the solvent mixture, and the final solution was filtered before use.

Method Validation Protocol

Justification of the settled HPLC system was approved ready permitting to the ICH procedures through deference to specificity, precision, robustness, linearity, accuracy, and force degradation studies.

Specificity

Specificity of the assay method is the capacity to evaluate the targeted analyte to be absent from the constituents, whose influence is expected to be present. The result could be seen from the chromatograms and the acceptance criteria will be the targeted analyte should not be interfered with the excipient compounds.

Accuracy

Accuracy of an analytical method is the nearness between the reference value and the observed value. The reference value can be found by determining the sample with a known concentration. The samples were prepared at three concentrations.

Practically no measurement process is ideal, therefore, the true or actual value cannot be exactly known in any particular measurement. The accepted true value for accuracy assessment can be assessed by analyzing a sample with a known concentration. The accuracy of an analytical method expresses the closeness of agreement between the value accepted either as a conventional true value or an accepted reference value and the value found.

Precision

Precision shows the proximity of observance between a streak of measurements obtained from a mutual sampling of the identical sample. The intra- and inter-day precision studies were performed by determining the response of three different concentrations (80, 90, and 100%), six times, and the results are shown as percentage relative standard deviation (% RSD).

Linearity

Linearity of an analytical method is determined to evaluate whether test results are directly proportional to the sample concentrations over a given range.

Robustness

Robustness of the method is the measurement of its capability to remain unaffected by small, but deliberate changes in method parameters, like flow rate, temperature, and pH. This value provides a sign of method reliability and consistency during normal usage.

Forced Degradation Studies

Force degradation studies provide measures to estimate the stability of tested drug samples during quantification. The stability of drug products during estimation can be affected by various parameters. This data may be useful while selecting proper formulation, package, proper storage conditions, and shelf life.

RESULTS AND DISCUSSION

Method Validation and Optimization

The proposed diagnostic philosophy was created to give a basic, reproducible, and fast RP-HPLC-UV technique for the concurrent recognition and evaluation of AMD and LIS. During the advancement of the explanatory system, a few chromatographic parameters were researched thinking about the two medications. Chromatographic conditions, for example, composition, pH, and flow rate of mobile phase just as the chromatographic column of examination were researched to acquire peaks with great partition, balance, and shape, together with satisfactory maintenance times.

A successful chromatography method was developed using the SunFire C8 column (4.6 × 150 mm, 5 µm) with gradient elution of the mobile phase composed of 85:25 potassium dihydrogen phosphate in water and methanol at a flow rate of 0.7 mL/min. The wavelength was set at 212 nm for the simultaneous estimation of AMD and LIS. The concurrent study of AMD and LIS, displayed respectable peaks parting and profile for all particles within an entire retention time of 20 minutes, as shown in Figure 2. The chromatographic conditions of the proposed HPLC method are shown in Table 1.

Method Validation

Specificity/Selectivity

In order to determine the selectivity of the created method, obtained chromatograms of standard and test samples of AMD and LIS were studied for certain parameters. No barrier or interference was recognized between the dissolvable parts with the chromatographic peaks beginning from the active elements, AMD with maintenance time of 5.1 minutes, and LIS with maintenance time of 10.5 minutes (Figure 2).

Linearity and Range

The linearity of the technique was controlled by developing position bends. Standard arrangements of AMD and LIS at various concentrations level (60, 80, 100, 120, and 140%) for assay and uniformity of dosage unit testing were utilized for this reason. Earlier to the infusion of the arrangements, the section was equilibrated for any event of 30 minutes with the manageable stage. Every estimation was completed in six duplicates to check the reproducibility of the indicator reaction at every complex level. The peak zones of the chromatograms were plotted against the groupings of AMD and LIS to get the position bends. The correlation coefficient (r^2) of 0.9994 > 0.99 for AMD and (r^2) of 0.998 > 0.99 for LIS, and calibration plots were prepared by plotting peak area against the corresponding concentration of AMD and LIS, as shown in Figure 3, and the results for linearity and retention time of AMD and LIS are shown in Table 2. The obtained correlation coefficients were ideal and showing that the method adopted was linear.

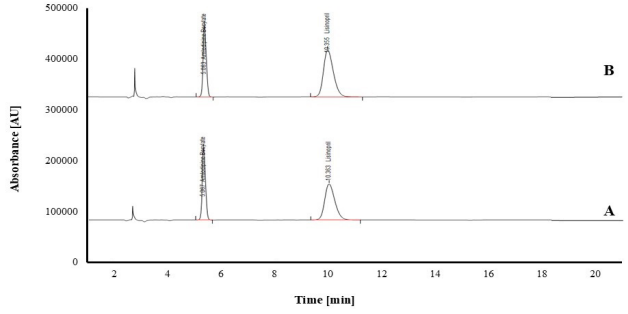

Figure 2: Chromatograms: **(A)** Standard solution, 0.05 mg/mL each of AMD (RT 5.003) and LIS (RT 10.355) at 212 nm; **(B)** Test solution, 0.05 mg/mL each of AMD (RT 5.007) and LIS (10.363) at 212 nm

Table 1: Chromatographic conditions of RP-HPLC method

Parameters	*Conditions*
Mobile phase	Buffer:methanol (85:15) (buffer prepared by dissolving 2.72 gm potassium dihydrogen phosphate in 1,000 mL of water, then the pH was adjusted to 7 through trimethylamine)
Column	SunFire C8 column (150 cm × 4.6 mm)
Method	Isocratic
Flow rate	0.7 mL/min
Column temperature	35ºC
Injection volume	50 µL/min
Wavelength	212 nm
Run time	20 min

Table 2: Linearity and retention time (RT) for the analysis of AMD and LIS

	Linearity	*Determination coefficient (r^2)*	*Linearity range (mcg/mL)*	*RT*
AMD	y = 16,192x + 37,861	0.9994	40 ~ 100	5.003
LIS	y = 26,527x + 37,861	0.998	30 ~ 70	10.355

Table 3: Result of accuracy of AMD and LIS

	Percentage recovery assay	*% RSD*	
Level (%)	*AMD LIS*	*AMD*	*LIS*
80	100.26 102.19 100.66 103.11 101.06 105.62	0.40	1.71
100	95.74 99.57 97.42 96.45 95.52 96.38	1.08	1.87
120	98.88 91.65 97.74 92.32 97.33 92.1	0.82	0.4

Table 4: Robustness of proposed HPLC method

	% RSD	
Parameters	*AMD*	*LIS*
Mobile phase ratio (85:15)		
75:25	0.04	0.05
95:5	0.07	0.16
pH of mobile phase (7)		
6.95	1.52	1.43
7.05	0.06	0.08
Wavelength (212 nm)		
210 nm	0.5	0.11
214 nm	0.67	0.11
Flow rate (0.7 mL/min)		
0.6 mL/min	0.04	0.13
0.8 mL/min	0.8	0.08
Column oven temperature		
25ºC	0.06	0.14
45ºC	0.09	0.08

Accuracy

The accuracy of the technique was decided by a popular addition technique. Three-stage of the solution (80, 100, and 120%) with analytical attention were prepared and percent recovery, general deviation, and RSD for every analyte (n = 5) were decided. The obtained data (Table 3) are indicating that percentage recovery of both substance (AMD and LIS) of all concentrations was in the range of 97.33 to 101.06, and RSD was lower than 2%, which is an ideal range of the parameter.

Robustness

The robustness of the technique was determined to evaluate the effect of moderate, however, measured variation of the chromatographic situations on the dedication of AMD and LIS. Robustness of the method was determined by changing five HPLC variables, like flow rate (0.6 and 0.8, mL min^{-1}), buffer solution ratio (95:5 and 75:25), wavelength for the detection of UV (210 and 214 nm), mobile phase pH (6.95 and 7.5), and column oven temperature (25 and 45ºC). The effect of these changes on the % RSD of both the substances (AMD and LIS) was determined. Obtained data, as shown in Table 4, indicates that the value of percentage standard deviation was within the range. So, it can be concluded that the developed method is robust and none of the variables possess significant effects on the method performance.

Table 5: Results from different stress conditions

	% degradation	
Stress condition	*AMD*	*LIS*
Acidic (5M HCl)	10.85	10.35
Alkaline (1M NaOH)	9.33	8.63
Oxidative (10% H_2O_2)	8.33	10.47

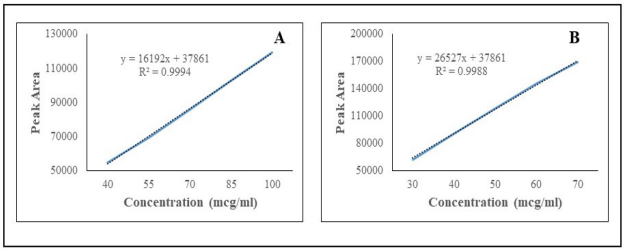

Figure 3: Standard calibration curve for **(A)** AMD and **(B)** LIS, by RP-HPLC

Forced Degradation Studies

All degradation investigates in solution was completed at a drug concentration for acid, basic, and oxidation degradation. All the degradation takes a look at samples, had been analyzed using a PDA detector with respective attention of test dilution. During the stress study, located that AMD and LIS labile to degradation in acid, base, and peroxide strain condition. The pressured degradation study revealed that no interference was observed from blank and placebo at the retention time of the principal peak and impurities. All peaks determined homogeneous and the peak purity data determined in the approval limit, is shown in Table 5.

CONCLUSION

The present RP-HPLC technique described the evaluation of AMD and LIS in the tablet dosage form. The after-effects of approval plans exhibited the great stability analysis method, which demonstrated the reliability of the proposed RP-HPLC strategy for the quantitative estimation of both the above said medicates in the oral dosage form.

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