Investigations and HPLC Assay of Model Formulations Containing Amlodipine Besylate and Lisinopril

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ABSTRACT

This paper describes the development and validation of a high-performance liquid chromatographic analytical procedure for simultaneous determination of Amlodipine Besylate (AML) and Lisinopril (LIZ) in model tablet formulations. The separation was achieved with a C_{18} (250 mm x 4.6 mm, 10 μm) column, at room temperature in an isocratic mode, with the mobile phase containing acetonitrile and 0.5 M sodium acetate buffer (25:75). The flow rate was 1.5 ml/min and the eluent was monitored at 215 nm. The selected chromatographic conditions were found to effectively separate Amlodipine Besylate and Lisinopril, with retention times of 6.67 min and 12.00 min, respectively. The method was validated for specificity, linearity, precision, accuracy, LOD and LOQ. The calibration curves were linear in the concentration range of 5.00-40.00 μg/ml for both AML and LIZ. The intra- and inter-day relative standard deviations for both the components were <2.0 %. The analytical procedure was applied in identification, purity and assay tests on model drug formulations. It was established that the developed analytical procedure was successfully used for routine analysis of AML and LIZ in model drug dosage forms without any interference from included excipients.

Keywords: Amlodipine Besylate, Lisinopril, liquid chromatography, validation, model tablet formulations.

INTRODUCTION

The goal of antihypertensive therapy is to abolish the risks associated with blood pressure elevation without adversely affecting quality of life. Drug selection is based on efficacy in lowering blood pressure and in reducing cardiovascular end points including stroke, myocardial infarction and heart failure. There has been a marked increase in the use of combinations of antihypertensive drugs with complementary mechanisms of action, with the aim of reducing blood pressure levels more rapidly and improving treatment compliance. Amlodipine Besylate, 2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-3-ethoxycarbonyl-5-methoxycarbonyl-6-methyl-1,4 dihydropyridine benzene sulphonate, is a dihydropyridine calcium-channel blocker that inhibits the transmembrane influx of calcium ions into vascular smooth muscles and cardiac muscles, which in turn affects their contractile process and results in reduced blood pressure. It is used in the treatment of hypertension and coronary artery diseases [1, 2]. Lisinopril is a drug of the angiotensin converting enzyme (ACE) inhibitor class primarily used in treatment of hypertension, congestive heart failure, and heart attacks and also in preventing renal and retinal complications of diabetes [2]. Its indications, contraindications and side effects are as those for all ACE inhibitors. It is designated chemically as *N*2-[(1*S*)-1-carboxy-3-phenylpropyl]-L-lysyl-L-proline. Certain successful attempts have been made for the determination of AML[3-12] and LIZ [13-32] in pharmaceuticals using different analytical techniques including spectrophotometry, spectrofluorimetry, fluorimetry, liquid chromatography, thin-layer chromatography, titrimetry, voltammetry, alone or in combination with other drugs. Some analytical methods for simultaneous estimation of both drugs have been recently reported[33-41]. The present study emphasizes on the determination of Amlodipine Besylate and Lisinopril in model drug tablets by using high performance liquid chromatography.

MATERIALS AND METHODS

Materials

Amlodipine besyalte and lisinopril were used as standards. HPLC grade acetonitrile was used to prepare the mobile phase. All other chemicals used for the chromatographic experiments were of a reagent grade. For the preparation of the model tablet formulations, amlodipine and lisinopril substances of Eur.Ph. grade were used. Ludipress (BASF), microcrystalline cellulose (Avicel PH 102), croscarmellose (Ac-Di-Sol), Calcium phosphate dibasic anhydrous and magnesium stearate were used as excipients in the preparation of the tablets. All used excipients were of an analytical grade.

Methods

Instrumentation and chromatographic conditions

Chromatographic separation was performed on a modular HPLC system LC-10A Shimadzu (Japan) comprising a LC-10A pump, a solvent degasser DGU-3A, a Rheodyne injector with 20 μl loop, a column oven CTO-10A, SPD-M10A UV detector with a fixed wavelength and

a communication bus module CBM-10A. A LiChrosorb C_{18} (250 mm x 4.6 mm, 10 μm) column was used as the stationary phase. The components were separated isocratically using a mobile phase consisting of acetonitrile and 0.5 M sodium acetate buffer (25:75) at a flow rate of 1.5 ml/min. The mobile phase was filtered through a 0.45 μm membrane filter and degassed. The analysis was carried out at a room temperature and the injection volume was 20 μl. The UV detector was set at 215 nm.

Preparation of reference solutions

Reference solution (a): The solution was prepared by dissolving 10.0 mg of accurately weighed Amlodipine besylate RS and 10.0 mg of Lisinopril RS in methanol in a 100.0 mL volumetric flask. Reference solution (b): The solution was prepared by diluting 10.0 mL of reference solution (a) with methanol into a 50.0 mL volumetric flask.

Sample preparation

The homogenized powder from twenty tablets with an average weight equivalent to 10 mg AML and 10 mg LIZ was transferred into a 100.0 ml volumetric flask. Approximately 70 ml methanol was added and the obtained mixture was sonicated for 20 min with intermittent shaking. The content was restored to room temperature and diluted to volume with methanol to furnish a stock test solution. The stock solution was filtered through a 0.45 μm Nylon syringe filter and 5.0 ml of the filtrate was diluted into a 25.0 ml volumetric flask to give a test solution containing 20.00μg/ml AML and 20.00 μg/ml LIZ.

Preparation of the model tablets

Model tablet formulations were produced by the method of direct compression. A single punch tablet press (EK0, Korsh, Germany) at 8 kN pressure and a set of 7 mm diameter standard concave tooling were used. The model tablet formulations contained 10 mg amlodipine besylate and 10 mg lisinopril. The tablets had a total weight of 150 mg.

Determination of the mechanical strength

The study was carried out by the progressive loading method according to Eur. Ph. 7.0 (2.9.8) using an Erweka type TBH 30 apparatus (Germany).

Determination of the friability

The study was performed according to Eur. Ph. 7.0 (2.9.7) using an Erweka type TAR 20 friabilator (Germany).

Determination of the disintegration time

The investigation was performed according to Eur. Ph 7.0 (2.9.1) in the basket-rack assemble using an Erweka, type ZT 3 apparatus (Germany).

In vitro drug dissolution studies

An Erweka DT 60, Hensenstmm (Germany) USP Apparatus 2 (paddle) was chosen to evaluate the drug release profiles). The dissolution test was carried out in 500 ml of water solution with pH 1.2, at a paddle speed of 50 rpm, at temperature 37 ± 0.5 °C. Five milliliter samples were withdrawn and filtrated through a 0.45 μm filter at predetermined intervals of 5, 10, 15, 30 and 45 minutes. The quantities of amlodipine and lisinopril dissolved in the samples were determined by the above described HPLC analytical procedure. The cumulative percentage of drug release was calculated and the average of six determinations was used in the data analysis. The statistical analysis of the dissolution data of the tablets showed a statistically significant difference (p < 0.05) in the t-test applied by using Origin Plot software.

RESULTS AND DISCUSSION

In this work, an LC method with UV detection for analysis of AML and LIZ in a model tablet formulation was developed and validated. From the chromatogram shown in **Fig. 1**, it is evident that, under the proposed chromatographic conditions, both analytes of interest are completely separated, which indicates that the method is selective and could be applied for their simultaneous identification and quantification.
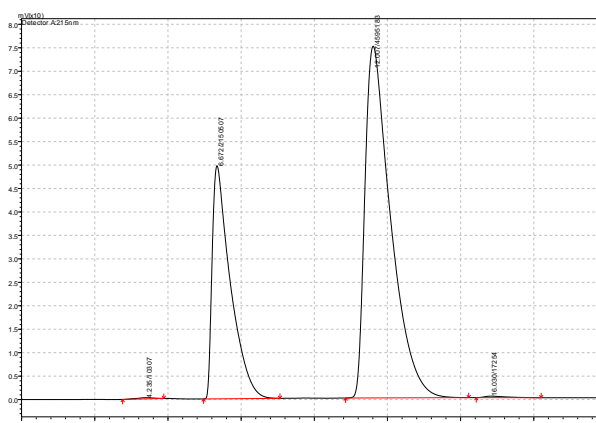

Figure 1: Chromatogram of Amlodipine Besylate RS and Lisinopril RS

Method validation

The proposed method was validated as per ICH guidelines[42] with respect to specificity, linearity, precision, accuracy, limit of quantitation (LOQ) and limit of detection (LOD).

Specificity

The specificity of the method was determined by checking the interference of the components against placebo. No interference was observed for any of the excipients of both drugs.

Calibration and linearity

Calibration curves were plotted in the range of 5.00-40.0 μg/ml for AML and 5.00-40.0 μg/ml for LIZ. The corresponding linear regression equations were

$y=158741.1x-2357.2$ with a squared correlation coefficient R^2 of 0.9998 for AML, and $y=65478.1x-3475.2$ with R^2 of 0.9998 for LIZ, respectively. Excellent correlation existed between the peak areas and the concentrations of both compounds.

Limit of quantitation and limit of detection

The limit of quantitation and the limit of detection were calculated from the standard deviations and slopes of the responses using a signal-to-noise ratio as per ICH guidelines [42]. The LOQs for AML and LIZ were found to be 0.5 µg/ml and 1 µg/ml, while the LODs were 0.1 µg/ml and 0.2 µg/ml, respectively.

Accuracy

The accuracy of the method was evaluated by using the standard addition technique, which was performed by adding known amounts of pure AML and LIZ to known concentrations of tablet powder and analysing by the proposed methods, in triplicate. The results presented in table 1 indicated good accuracy and showed no interference from tablet excipients.

Precision

The intraday precision (repeatability) was determined by analyzing two concentrations of AML (10, 20 µg/mL) and LIZ (15, 30 µg/mL), in triplicate, using the proposed methods. The inter day precision (reproducibility) was determined by repeating three times on three different days the analysis of two different concentrations (10:15, 20:30 µg/mL) for both drugs. The values of % RSD (table 2) for AML and LIZ were found to be in the range from 0.35 to 0.94 indicating good repeatability and reproducibility of the analytical procedure.

Table 1: Recovery studies of AML and LIZ

Drug	Amount taken (µg/ml)	Amount added (µg/ml)	Amount recovered ± SD* (µg/ml)	% RSD
AML	10.00	2.50	12.42±0.06	0.48
		5.00	14.78±0.05	0.34
		7.50	17.31±0.08	0.46
LIZ	10.00	2.50	12.67±0.05	0.39
		5.00	15.08±0.07	0.46
		7.50	17.69±0.09	0.51

*Average value of three determinations, RSD is relative standard deviation

Table 2: Precision of the method

Precision	Amount taken (µg/ml)		% Mean*		% RSD	
	AML	LIZ	AML	LIZ	AML	LIZ
Intra day	10.00	15.00	99.54	100.1	0.37	0.79
Intra day	20.00	30.00	99.70	99.52	0.68	0.71
Inter day	10.00	15.00	100.2	99.56	0.67	0.35
Inter day	20.00	30.00	99.76	100.1	0.81.	0.94

*Mean of three determinations

Preparation of Model Tablet Formulations

Two model compositions based on different excipients were prepared by the method of direct compression with 8 kN pressure force. The obtained model formulations were with the required uniformity of mass, mechanical strength 80-90 N and low friability – under 1%. The disintegration time was in the range of 2-5 minutes for the both models. The compositions and properties of the model formulations are given in table 3.

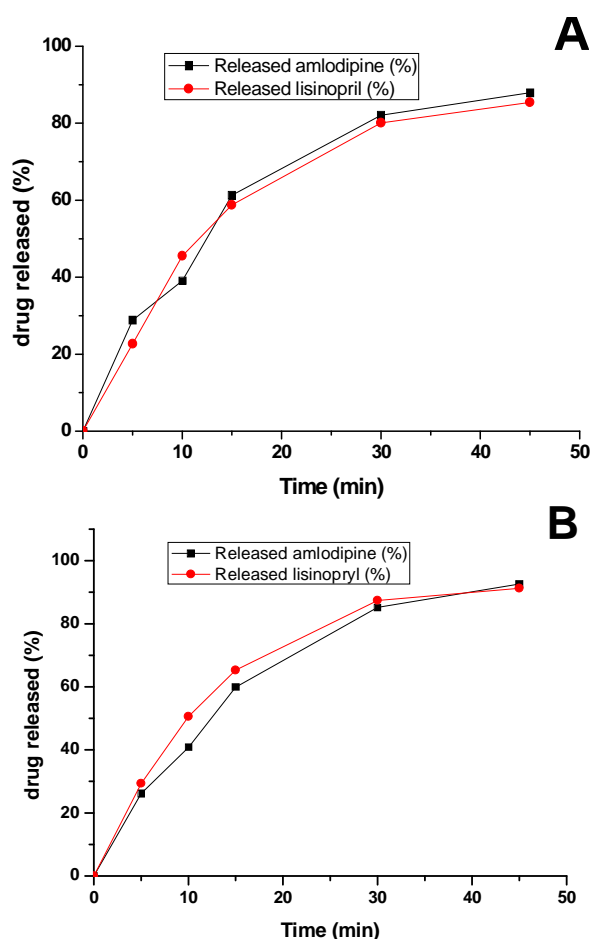### Release kinetics of amlodipine and lisinopril from model drug tablets

The release profiles of AML and LIZ from the model tablets at pH 1.2 are presented in Fig. 2.

From the figure it can be clearly seen that the quantity of drugs released at the thirtieth minute from both models was more than 80 % of the contained amlodipine and lisinopril. The kinetic of drug release is very close to a first order kinetic: for M1 (R for amlodipine is 0.985 and for lisinopril – 0.996), for M2 (R for amlodipine is 0.995 and for lisinopril – 0.998). The results indicate that there was no significant difference in the release kinetics of drugs from the both model tablets – the factor of similarity (f_2) for amlodipine was 65.9 and for lisinopril - 60.8.

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Table 3: Composition and characteristics of model tablet formulations

Ingredients (mg)	Formulation code	
	M1	M2
Lisinopril	10	10
Amlodipine Besylate	10	10
Ludipress BASF	138.8	
Croscarmellose (Ac-Di-Sol)	-	7
Calcium phosphate dibasic anhydrous	-	101.8
Microcrystalline cellulose (Avicel pH 102),	-	30
Magnesium stearate	1.2	1.2
Properties		
Weight of tablet (mg)	150 ± 1.2	150 ± 1.7
Mechanical strength (N)	90 ± 5.4	80 ± 3.9
Friability (%)	0.5	0.5
Disintegration time (min)	5	2

Figure 2: Release kinetics of amlodipine and lisinopril from model formulations: (A) Model M1; (B) Model M2.

CONCLUSION

The validated RP-LC method developed here proved to be simple, specific, accurate and precise. It can be successfully used for routine analysis of amlodipine and lisinopril in combined dosage forms without any interference from common excipients.

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