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Research Article

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Development and validation of RP-HPLC method for simultaneous determination of Amlodipine Besylate and Hydrochlorothiazide in pharmaceutical dosage form

Boyka G. Tsvetkova* and Lily P. Peikova

Medical University – Sofia, Faculty of Pharmacy, Department of Pharmaceutical chemistry, 2 Dunav str., 1000 Sofia, Bulgaria

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ABSTRACT

A simple, rapid and prcise method was developed for the simultaneous determination of Amlodipine Besylate (AML) and Hydrochlorothiazide (HTZ) in tablet dosage form. Analysis was performed on a C8 (250 mm x 4.6 mm, 5 µm) column with acetonitrile:0.5 M phosphate buffer (pH 3) 60:40 (v/v) as mobile phase, a flow rate 1.0 ml/min and column temperature 30ºC. Quantitation was achieved with UV detection at 272 nm. Both the drugs were well resolved on the stationary phase and the retention times were found to be 3.09 min for AML and 7.41 min for HTZ. The calibration curves were linear in the concentration range of 5.00-40.00 µg/ml for AML and 12.50-100.0 µg/ml for HTZ. Intra- and inter-day relative standard deviations for both the components were <2.0%. The method was found to be accurate, with recoveries in the range 99.28 % – 100.3 %. The method proposed proved to be specific, rapid and accurate for the quality control of both drugs in pharmaceutical preparation.

Key Words: liquid chromatography, validation, amlodipine besylate, hydrochlorothiazide, tablet dosage form

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INTRODUCTION

Fixed-dose combination of antihypertensive drugs can simplify dosing regimens, improve compliance, improve hypertension control, decrease dose-dependent side effects and reduce cost as the first-line treatment of hypertension [1]. These potential advantages make it recommendable for the combination antihypertensive therapy to be used as initial treatment, particularly in patients with target-organ damage or more severe initial hypertension [2, 3]. 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 [4, 5]. Hydrochlorothiazide, 6-chloro-1,1-dioxo-3,4-dihydro-2H-1,2,4-benzothiazine-7-sulphonamide, is a thiazide diuretic that increases sodium and chloride excretion by distal convoluted tubule [5]. Literature survey reveals the availability of several methods for determination of Amlodipine Besylate [6-23] and Hydrochlorothiazide [24-36] includes spectrophotometry, liquid chromatography, thin-layer chromatography, potentiometry, voltammetry as alone or in combination with other drugs. Some analytical methods for simultaneous estimation of both drugs have been recently reported [37-40]. Present study emphasizes on the determination of Amlodipine Besylate and Hydrochlorothiazide in their combined dosage form by high performance liquid chromatography [41].

EXPERIMENTAL SECTION

Reagents and chemicals

Tablets were purchased from local market each containing 5 mg of AML and 12.5 mg of HTZ. AML and HCT are available in the ratio of 1:2.5 respectively in the formulation and were used in same ratio for preparation of calibration curves. Amlodipine Besylate RS and Hydrochlorothiazide RS were used as standards and were purchased from Sigma-Aldrich (St. Louis, MO, USA). HPLC grade acetonitril was used to prepare the mobile phase. All other chemicals used for the chromatographic experiments were of a reagent grade.

Instrumentation and chromatographic conditions

Chromatographic separation was performed on a modular HPLC system LC-10AShimadzu (Japan) comprising a LC-10A pump, solvent degasser DGU-3A, Rheodyne injector with 20 µl loop, column oven CTO-10A, SPD-M10A UV detector with fixed wavelength and communication bus module CBM-10A. Separation was achieved isocratically with a LiChrosorb C 8, 250 mm x 4.6 mm, 5 µm column eluted with a mixture of acetonitrile:0.5 M phosphate buffer (pH 3) (60:40 *v/v*) as the mobile phase at flow rate of 1 ml/min. The mobile phase was filtered through a 0.45 µm membrane filter and degassed. Detection was carried out by absorbance at 272 nm. The analysis was carried out at column temperature 30ºC and injection volume was 20 µl.

Preparation of reference solutions

Reference solution (a): The solution was prepared by dissolving 10.0 mg of accurately weighed Amlodipine Besylate RS and 25.0 mg Hydrochlorothiazide 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

Twenty tablets were weighed accurately and a quantity of tablet powder equivalent to 10 mg AML and 25 mg HTZ was weighed and dissolved in the 70 mL methanol with the aid of ultrasonication for 10 min. The content was diluted to 100 mL with methanol to furnish a stock test solution. The stock solution was filtered through a 0.45 µm Nylon syringe filter and 10.0 mL of the filtrate was diluted into a 50.0 mL volumetric flask to give a test solution containing 20 µg/mL AML and 50 µg/mL HTZ.

RESULTS AND DISCUSSION

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

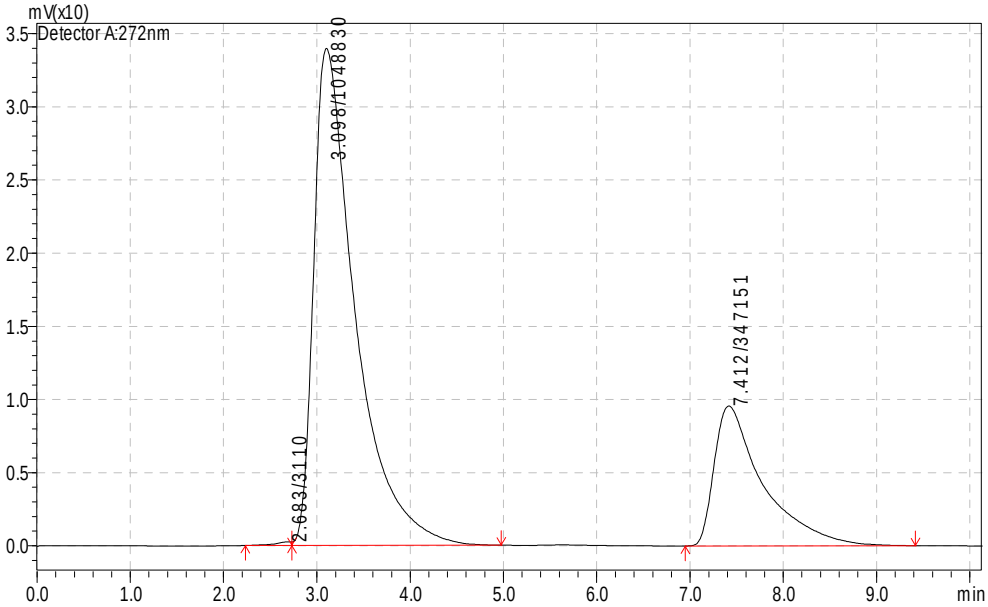

Figure 1: Chromatogram of Amlodipine Besylate RS and Hydrochlorothiazide RS

Method validation

The proposed method was validated as per ICH guidelines [47] 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.

Linearity

Under the experimental conditions described above, linear calibration curves for AML and HTZ were constructed with five concentration level each. Peak area and concentration of each drug substance was subjected to regression analysis to calculate the regression equations and the correlation coefficients. Calibration plot data slope (a), intrercept (b), and correlation coefficients (R^2) were listed in table 1.

Limits of quantitation and limits of detection

The limit of detection (LOD) was calculated to be three times the standard deviation of baseline noise from analysis of each compound. The limit of quantitation (LOQ) was measured as the lowest of analyte that could be reproducibly quantified above the baseline noise, i.e. for which duplicate injection resulted in an RSD ≤ 2%. The values for sensitivity of the method were presented in table 1.

Table 1: Regression analysis of calibration curves

Drugs	AML	HTZ
Concentration range (µg/mL)	5.00-40.00	12.5-100.0
Slope	155454.5	112613.8
Intercept	1610.5	1324.1
Correlation coefficient (R^2)	0.9998	0.9988
LOQs (µg/mL)	0.50	1.00
LODs (µg/mL)	0.10	0.20

Accuracy

Accuracy of the proposed method was determined using recovery studies. The recovery studies were carried out by adding different amounts (50%, 100%,150%) of the pure drug to the pre-analysed formulation. The solutions were prepared in triplicates and the % Recovery was calculated. The recovery study results ranged from 99.32 to 100.3 % for AML and from 99.28 to 100.2 for HTZ, respectively. Recovery values were close to 100 % and % RSD not more than 2 which indicated that the method was accurate. Results of recovery investigations were reported in table 2**.**

Table 2. Recovery studies of AML and HTZ

Amlodipine besylate				
Labelled claim (mg)	**Level of addition (mg)**	**Amount of pure drug added (mg)**	**% Recovery***	
5	50	2.5	100.3	
5	100	5	99.54	
5	150	7.5	99.32	
Statistical analysis			Mean	99.72
			SD	0.51
			%RSD	0.51
Hydrochlorothiazide				
Labelled claim (mg)	**Level of addition (mg)**	**Amount of pure drug added (mg)**	**% Recovery***	
10	50	5	99.28	
10	100	10	100.2	
10	150	15	99.57	
Statistical analysis			Mean	99.68
			SD	0.47
			%RSD	0.47

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

Precision

The precision of the method was demonstrated by inter-day and intra-day variation studies. In the intraday studies, six repeated injections of standard solutions were made and the response factor of drug peaks and percentage RSD were calculated. In the inter-day variation studies, six repeated injections of standard solutions were made for three consecutive days and response factor of drugs peaks and percentage RSD were calculated. From the data obtained, the developed method was found to be precise. The results of precision study are listed in table 3.

Table 3. Precision of the method

Parameters	Intraday		Interday					
	AML	HTZ	AML			HTZ		
			Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
Average	1324560	351452	1122389	1141976	1153756	389437.2	358670	344466.3
SD	4965.1	1925.6	4168.8	4754.9	4975.3	4212.4	3611.4	3388.8
% RSD	0.37	0.55	0.37	0.42	0.43	1.08	1.01	0.98

CONCLUSION

The proposed HPLC method provides simple, accurate and reproducible quantitative analysis for the simultaneous determination of amlodipine besylate and hydrochlorothiazide in tablet dosage forms. This method was validated as per ICH guidelines. The proposed method is suitable for the quality control determination of both drugs in ordinary laboratories.

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