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Analytical Method Development and Validation of Amlodipine and Benazepril hydrochloride in combined dosage form by RP-HPLC

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ABSTRACT

A simple, specific, accurate and precise reverse phase high pressure liquid chromatographic method has been developed for the simultaneous determination of Amlodipine and Benazepril hydrochloride from combined dosage form by reverse phase C18 column (Phenomenex C18, 5µ, 250mm x 4.6mm). The sample was analysed using Triethylamine: Acetonitrile: Methanol in the ratio of 50:25:25(pH adjusted to 3.0 with Orthrophosphric acid) as a mobile phase at a flow rate of 2.0ml/min and detection at 235nm. The retention time for Amlodipine and Benazepril hydrochloride was found to be 16.999 and 12.550 min respectively, and recoveries from combined dosage form were between 98 and 102%. The method can be used for estimation of combination of these drugs in combined dosage form.

Keywords: Amlodipine besylate, Benazepril hydrochloride, RP-HPLC.

1. INTRODUCTION

Amlodipine (as besylate, mesylate or maleate) is a long-acting calcium channel blocker (dihydropyridine class) used as an anti-hypertensive and in the treatment of angina[1]. Like other calcium channel blockers, Amlodipine acts by relaxing the smooth muscle in the arterial wall, decreasing peripheral resistance and hence reducing blood pressure; in angina it increases blood flow to the heart muscle. Benazepril hydrochloride is a medication used to treat high blood pressure (hypertension)[2], congestive heart failure, andchronic renal failure. Upon cleavage of its ester group by the liver, benazepril is converted into its active form benazeprilat, a non-sulfhydryl angiotensin-converting enzyme (ACE) inhibitor. Literature survey reveals the availability of several methods for estimation of both Amlodipine besylate and Benazepril hydrochloride includes UV[3-8], HPLC as alone or in combination with other drugs[9-11]. No method has been reported for the estimation of Amlodipine and Benazepril hydrochloride in combined dosage form. Present work emphasizes on the quantitative estimation of Amlodipine and Benazepril hydrochloride in their combined dosage form by RP-HPLC.

A High Performance Liquid Chromatography system, with LC solutions data handling system (WATERS ALLIANCE 2695 Separation module) with an auto sampler

was used for the analysis. The data was recorded using WATERS EMPOWER software. The purity determination performed on a stainless steel column 250mm long, 4.6mm internal diameter filled with Octadecyl silane chemically bonded to porous silica particles of 5µm diameter (Phenomenex C18, 5µ, 250mm x 4.6mm). Optimized chromatographic conditions are listed in Table 1.

2. MATERIALS AND METHODS

Pure samples of Amlodipine and Benazepril hydrochloride were obtained from Rakshi drugs Pvt.Ltd. and Shanpur Pharma chem. respectively for the estimation of Amlodipine and Benazepril hydrochloride in commercial formulations. HPLC grade Orthrophosphric acid, Acetonitrile and Methanol were procured from Qualigens fine chemicals. High pure water prepared by using Millipore Milli Q plus purification system.

2.1. Preparation of Standard stock solution

Solution (A):

Weighed accurately 62.5mg of Amlodipine besylate working reference standard and transferred carefully in to a 50ml volumetric flask. Added 35ml of mobile phase and sonicated for 15min, cooled to room temperature and diluted 50ml with mobile phase.

Solution (B):

Weighed accurately 25mg of Benazepril hydrochloride working reference standard and transferred carefully in to a 50ml volumetric flask. Added 35ml of mobile phase and sonicated for 15min, cooled to room temperature and diluted 50ml with mobile phase.

2.2. Mixtured standard solution

Diluted 5ml of Solution (A) and 5ml Solution (B) to 50ml with mobile phase.

2.3. Preparation of Sample solution

Weighed and finely powdered not less than 20 tablets. Transferred an accurately weighed portion of the powder equivalent to about 5mg to 100ml volumetric flask, added 70ml of mobile phase. Sonicated for 15min and cooled to room temperature. Diluted to 100ml with mobile phase. Mixed well and filtered through Whatman No.1 filter paper. Discarded first few ml of the filtrate. Injected separately 20µl of the standard preparation in to the equilibrated HPLC system in 5 replicate and measured the response of the major peak due to Amlodipine besylate and Benazepril hydrochloride. Then injected separately 20µl of the sample preparation in to duplicate and measured the response of the major peak due to Amlodipine besylate and Benazepril hydrochloride. And calculated the content of Amlodipine besylate and Benazepril hydrochloride.

2.4. Validation of the Method

The method was validated in terms of linearity, accuracy, precision and specificity of the sample applications. The linearity of the method was investigated by serially diluting the stock solutions of Amlodipine besylate, Benazepril hydrochloride and measured the absorbance at 235nm. Calibration curves where constructed by plotting the area against the concentration. Amlodipine shows the linearity in the concentration range from 80-120 µ/ml with correlation coefficient of 0.9999 and Benazepril hydrochloride shows the linearity in the concentration range from 200-300 µ/ml with correlation coefficient of 0.9998. Recovery studies were carried out to study the accuracy of the proposed method and ascertained by standard addition method. A known amount of drug was added to preanalysed tablet powder, at three level and the percentage recoveries were calculated. Precision was found to be lower than 1%. Ruggedness of the proposed method was determined by analysis of aliquots from homogenous slot by different analysts using similar operational and environmental conditions. Specificity study was performed by keeping the sample under various stressed conditions as at 600C and at 500C by adding 1ml of 0.1N HCl, 0.1N NaOH, 3% H2O2, exposing with UV light at 265nm[12-14].

3. RESULTS AND DISCUSSION

3.1. Estimation

A RP-HPLC method was developed for the simultaneous estimation of Amlodipine and Benazepril hydrochloride in combined dosage forms, which can be conveniently employed for routine quality control in pharmaceutical dosage forms. The chromatographic conditions were optimized in order to provide a good performance of the assay. The standard and sample solutions were prepared and chromatograms were recorded. The peak area ratios of standard and sample solutions were calculated. The assay procedure was repeated for 6 times and mean peak area, mean peak area ratio, mean weight of standard drugs, mean weight of sample taken for assay were calculated. The percentages of individual drugs found in formulations, mean and relative standard deviations in formulation were calculated. The result of analysis shows that the amount of drugs present in the formulation has a very good correlation with the label claim of the formulation.

3.2. Validation of the method

The accuracy of the method was determined by recovery experiments. A known quantity of the pure drug was added to the pre-analyzed sample formulation at 80%, 100% and 120% levels. The recovery studies were carried out 6 times of each level and the percentage recovery and mean of the percentage recovery were calculated and given in Table 3. From the data obtained, it was observed that the recoveries of standard drugs were found to be accurate and within the specified limits. The precision of the method was determined by studying repeatability and reproducibility. The area of drug peaks and percentage relative standard deviation were calculated. The results revealed that the developed method was found to be reproducible in nature. The standard drug solutions in varying concentrations ranging from 80 to 120 % of the targeted level of the assay concentration were examined by the assay procedure. Amlodipine and Benazepril hydrochloride were found to be linear in the range of 80 to 120 mg/ml and 200-300 mg/ml respectively. The slope, intercept and correlation coefficient values were also calculated. The correlation coefficient of Amlodipine and Benazepril hydrochloride were found to be 0.9999 and 0.9998 respectively. The calibration curves were plotted as peak area Vs concentration of the standard solutions. The calibration graph shows that linear response was obtained over the range of concentrations used in the assay procedure. These data demonstrates that the methods have adequate sensitivity o the concentration of the analytes. The range demonstrates that the method is linear outside the limits of expected use. The additional peaks were observed in the chromatogram of the formulation, which may be due to excipients present in the formulation. These peaks do not interfere with the standard peaks, which clearly confirm the assay method was found to be highly specific. The LOD and LOQ of the developed method were determined by analyzing progressively low concentration of the standard solutions using the develop methods. The LOD is the smallest concentration of the analyte that gives a measurable response (signal to noise ratio of 3.3). LOD of Amlodipine and Benazepril hydrochloride were found to be 0.80mg/ml and 3.40mg/ml respectively. The LOQ is the smallest concentration of the analyte, which gives response that can be accurately quantified (signal to noise ratio of 10). The LOQ of Amlodipine and Benazepril hydrochloride were found to be 2.43 mg/ml and 10.31 mg/ml respectively. The system suitability studies were performed for the standard solutions and were presented in Table 2. The values obtained demonstrated the suitability of the system for the analysis of the above drug combination. From the above experimental data results and parameters it was concluded that the developed RP-HPLC method has the following advantages. The standard and sample preparation requires less time. No tedious

Table 1: Optimized Chromatographic conditions

Parameter	Optimized condition
Instrument	WATERS ALLIANCE 2695
Column	Phenomenex C18,5µ,250mm x 4.6mm
Mobile phase*	Triethylamine:Acetonitrile:Methanol(50:25:25) pH 3.0 (dil.Orthophosphoric acid)
Flow	rate 2.0ml/min
Detection	235nm
Injection volume	20µl
Temperature	Ambient

*Filtered through a 0.45µ membrane filter (Millipore) degassed and sonicated.

Table 2: System Suitability Parameters

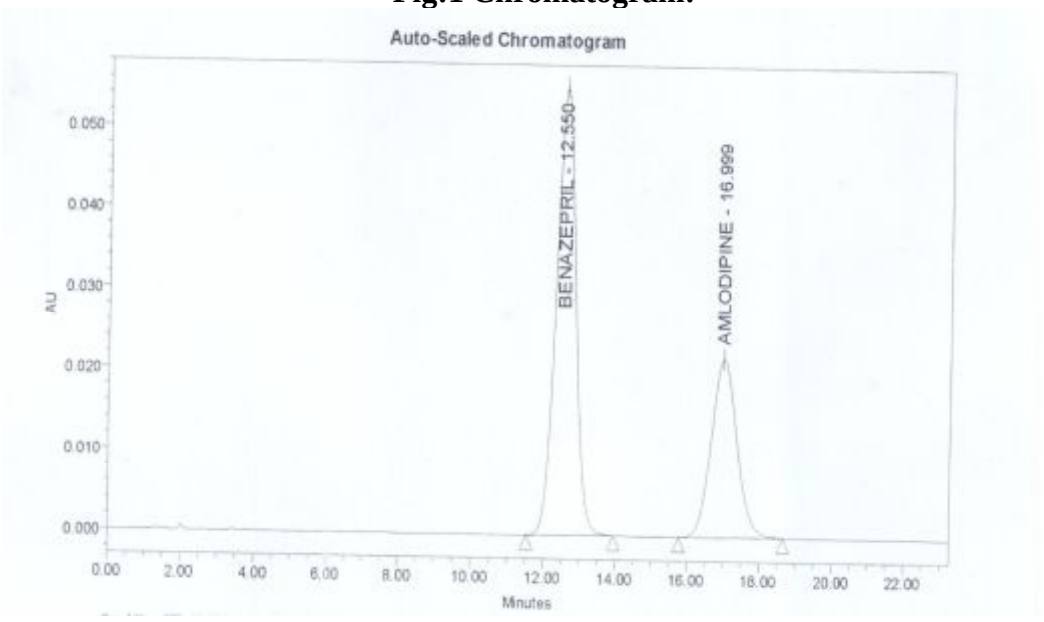
Parameter	Amlodipine besylate	Benazepril hydrochloride
Theoretical plates	4328.04	2919.54
Resolution	-	9.5
Tailing factor	1.4958	1.3601
LOD (µg/ml)	0.80mcg/ml	3.4mcg/ml
LOQ (µg/ml)	2.43mcg/ml	10.31mcg/ml

Table 3: Analysis of Formulation and Recovery studies

Drug	Label claim (mg/ml)	*Estimation	**Recovery	
		mg/tablet	Amount added(µg/ml)	% recovery
Amlodipine besylate	5mg	4.980	3.90	98.48%
			4.947	99.90%
			5.90	100.16%
Benazepril hydrochloride	12.5mg	12.41	9.858	99.37%
			12.27	98.95%
			14.70	98.80%

*mean (%RSD) of five observations, **mean (%RSD) of three determinations.

Fig:1 Chromatogram:



extraction procedure was involved in the analytical process. Ø Suitable for the analysis of raw materials. Run time required for recording chromatograms were less than 15 times. Hence, the chromatographic method developed for Amlodipine and Benazepril hydrochloride were found to be simple, precise, accurate and cost effective and it can be effectively applied for routine analysis in research institutions, quality control department in industries, approved testing laboratories, bio-pharmaceutical and bio-equivalence studies and in clinical pharmacokinetic studies in near future.

4. CONCLUSSION

The proposed RP-HPLC method for the simultaneous estimation of Amlodipine and Benazepril hydrochloride in combined dosage forms is accurate, precise, linear, rugged, roubust, simple and rapid. Hence the present method is suitable for the quality control of raw materials and formulations.

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