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Stability indicating RP-HPLC method for simultaneous determination of amlodipine and benazepril hydrochloride from their combination drug product

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Abstract

A stability indicating reversed-phase HPLC method has been developed and subsequently validated for simultaneous estimation of amlodipine (AM) present as amlodipine besylate (AB), and benazepril hydrochloride (BH) from their combination product. The proposed RP-HPLC method utilizes a Zorbax SB C18, 5 μm, 250 mm × 4.6 mm i.d. column, mobile phase consisting of phosphate buffer and acetonitrile in the proportion of 65:35 (v/v) with apparent pH adjusted to 7.0, and UV detection at 240 nm using a photodiode array detector. AB, BH, and their combination drug product were exposed to thermal, photolytic, hydrolytic, and oxidative stress conditions, and the stressed samples were analysed by the proposed method. Peak homogeneity data of AM and BH peaks obtained using photodiode array detector, in the stressed sample chromatograms, demonstrated the specificity of the method for their estimation in presence of degradants. The described method was linear over a range of 6–14 μg/ml for AM and 12–28 μg/ml for BH. The mean recoveries were 99.91 and 100.53% for AM and BH, respectively. *F*-test and *t*-test at 95% confidence level were used to check the intermediate precision data obtained under different experimental setups; the calculated value was found to be less than critical value.
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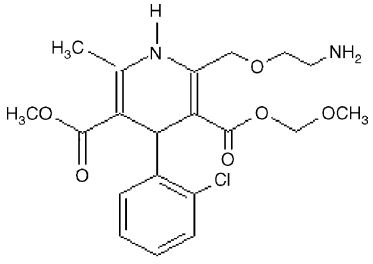
Keywords: Stability indicating RP-HPLC; Amlodipine; Benazepril; Diode array detection

1. Introduction

Amlodipine besylate (AB) is the benzene sulfonate (besylate) salt of amlodipine (AM), which is a dihydropyridine calcium channel blocker. AM is a calcium antagonist 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 angina. Benazepril hydrochloride (BH) and its active metabolite benazeprilat are non-sulfhydryl angiotensin converting enzyme (ACE) inhibitors. ACE is a peptidyl dipeptidase that catalyses the conversion of angiotensin I into angiotensin II, a vasoconstrictor substance. As BH inhibits ACE, it ultimately results in reduction in vasoconstriction and is used in the treatment of hypertension. The combination therapy of AM and BH was shown to be superior in lowering systolic and diastolic blood pressures when compared with either of the monotherapy regimens [1]. Combination therapy also has significantly fewer dose-dependent adverse experiences [2] as against high-dose calcium antagonist monotherapy. Combination drug products of AM and BH are hence widely marketed and used in the treatment of hypertension and cardiac disorders.



Amlodipine (AM)

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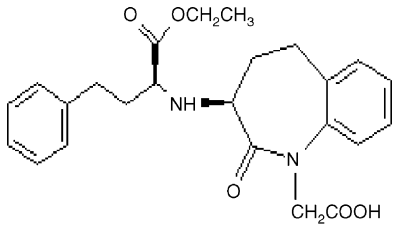

Benazepril (BH)

Stability testing forms an important part of the process of drug product development. The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity, and light, and enables recommendation of storage conditions, retest periods, and shelf lives to be established. The two main aspects of drug product that play an important role in shelf life determination are assay of active drug, and degradants generated, during the stability study. The assay of drug product in stability test sample needs to be determined using stability indicating method, as recommended by the International Conference on Harmonization (ICH) guidelines [3] and USP 26 [4]. Although stability indicating methods have been reported for assay of various drugs in drug products, most of them describe assay procedures for drug products containing only one active drug substance. Only few stability indicating methods are reported for assay of combination drug products containing two or more active drug substances. The objective of this work was to develop an analytical LC procedure, which would serve as stability indicating assay method for combination drug product of AB and BH.

Both the drugs AM and BH are not official with USP 26. EP 2002 [5] describes an HPLC method for determination of AM, but does not involve simultaneous determination of BH. Detailed survey of literature for AM revealed several methods based on different techniques, viz. HPLC [6–13], HPTLC [14–16], OPLC [17], SFC [18], UV spectrophotometry [19–31], and MEKC [32] for its determination from pharmaceuticals. Similarly, survey of literature for BH revealed methods based on HPLC [33–40], HPTLC [41], UV spectrophotometry [42–47], eletroanalytical techniques [48,49], and CE [50–52] for its determination from pharmaceuticals. Only one method [13] has been reported for simultaneous determination of AM and BH, which describes an RP-HPLC procedure using C18 column, but this method lacks stability indicating nature. A stability indicating LC method with gradient elution has been reported for determination of BH [35]. None of the reported analytical procedures describe a stability indicating method for simultaneous determination of AM and BH in presence of their degradants.

This manuscript describes the development and subsequent validation of a stability indicating isocratic reversed-phase HPLC method for simultaneous determination of AM and BH in presence of their degradants. To establish the stability indicating nature of the method, forced degradation of drug substances and drug product was performed under stress conditions (thermal, photolytic, acid and basic hydrolytic and oxidative), and stressed samples were analysed by the proposed method. The proposed LC method was able to separate both drugs from degradants generated during forced degradation studies. The linearity of response, accuracy and intermediate precision of the described method for assay of AM and BH has been checked.

2. Experimental

2.1. Chemicals and reagents

AB and BH working standards were generous gifts from Wockhardt Ltd. (Aurangabad, India) and Novartis Pvt. Ltd. (Mumbai, India), respectively. Combination product of AB and BH (Label claim: amlodipine 5 mg, as amlodipine besylate, and benazepril hydrochloride 10 mg), Amace-BP tablets (Systopic Laboratories Ltd.) were purchased from the market. Acetonitrile, methanol, potassium dihydrogen phosphate, sodium hydroxide, hydrochloric acid and hydrogen peroxide were from Qualigens Fine Chemicals (Glaxo Ltd.). Quinine monohydrochloride dihydrate was obtained from HiMedia Chemicals (Mumbai, India).

2.2. HPLC instrumentation and conditions

The HPLC system consisted of Thermo Separation Products 'P1000' pump, 'AS1000' autosampler and '6000LP' photodiode array detector. The chromatographic separations were performed using Zorbax SB C_{18}, 5 μm, 250 mm × 4.6 mm i.d. column, maintained at 28 °C using column oven, eluted with mobile phase at the flow rate of 1.0 ml/min. The mobile phase consisted of 0.05 M potassium dihydrogen phosphate buffer–acetonitrile (65:35, v/v), apparent pH adjusted to 7.0 with 1.0N potassium hydroxide solution, filtered through 0.45 μm nylon filter and degassed in ultrasonic bath prior to use. Measurements were made with injection volume 25 μl and ultraviolet (UV) detection at 240 nm. For analysis of forced degradation samples, the photodiode array detector was used in scan mode with a scan range of 220–400 nm and desired peak coverage of 100%. The output signal was integrated using PC1000 software (Thermo Separation Products). Peak homogeneity was expressed in terms of peak purity values, and was obtained directly from the spectral analysis report obtained using the above-mentioned software.

2.3. Standard and sample preparation

The standard stock solutions 1000 μg/ml each of AM (as AB) and BH were prepared separately by dissolving working standards in small proportion of methanol and later diluted to desired volume with mobile phase. Standard calibration solutions of AM and BH having concentration in the range of

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