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Journal of Pharmaceutical and Biomedical Analysis 39 (2005) 147–155
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
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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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Received 3 March 2004; received in revised form 3 April 2005; accepted 5 April 2005
Available online 6 June 2005

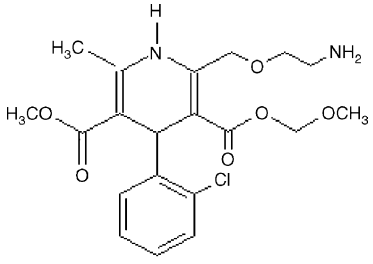
Abstract

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.

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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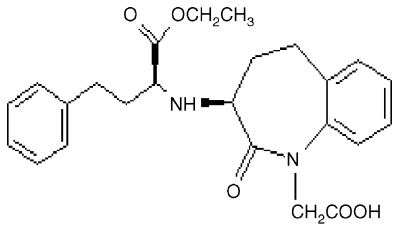
0731-7085/$ – see front matter © 2005 Published by Elsevier B.V.
doi:10.1016/j.jpba.2005.04.001



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

6–14 and 12–28 μg/ml, respectively, were prepared by diluting stock solution with mobile phase.

2.4. Analysis of dosage form

Ten tablets were weighed, their mean weight determined, and crushed in mortar. An amount of powdered mass equivalent to one tablet content was transferred into a 50 ml volumetric flask containing 10 ml of methanol, mechanically shaken for 10 min, ultrasonicated for 5 min, and then diluted to volume with mobile phase (sample stock solution). About 10 ml of sample stock solution was centrifuged at 10,000 rpm, and 5 ml aliquot diluted to 50 ml with mobile phase (sample solution). A small portion of sample solution was filtered through 0.45 μm nylon filter and used for injection on HPLC.

2.5. Procedure for forced degradation study

Forced degradation of each drug substances and the drug product was carried out under thermolytic, photolytic, acid/base hydrolytic and oxidative stress conditions. Thermal and photodegradation of drug substances and drug product was carried out in both solid and solution state. Solutions were prepared by dissolving drug substance or drug product in small volume of methanol and later diluted with either distilled water, aqueous hydrochloric acid, aqueous sodium hydroxide, or aqueous hydrogen peroxide solution, to achieve a concentration of 200 μg/ml each of AM and BH. After the degradation these solutions were diluted with mobile phase to achieve a concentration of 20 μg/ml each of AM and BH. Based on the labeled strength of AM and BH in tablets, the nominal concentration of AM and BH in its solution was 10 and 20 μg/ml, respectively.

For thermal stress, samples of drug substances and drug product were placed in a controlled-temperature oven at 80 °C for 48 h. Solutions of drug substances and drug product were also kept at 80 °C for 48 h.

For photolytic stress, samples of drug substances and drug product, both in solid and solution state, were irradiated with UV radiation having peak intensities at 254 and 366 nm. The UV dose from the lamp having peak intensity at 366 nm was measured using a quinine monohydrochloride (2% solution in water) chemical actinometer, as mentioned in photostability-testing guidelines of ICH [53]. The ICH guidelines state the minimum desired exposure as 200 W/m^2, which corresponds to a change in absorbance of 0.5 AU of quinine actinometer at 400/nm. This change was observed in 24 h of irradiation. In this initial photolytic stress-testing experiment, AM showed significant amount of photodegradation in solution state. A second photolytic stress testing experiment with greater irradiation time, 48 h, was performed to establish the specificity of assay method even in presence of any secondary degradants. A tightly wrapped quartz cell containing the quinine solution was kept in the UV chamber during the entire period of exposure as thermal control. Absorbance of the wrapped cell measured after the completion of exposure period did not show any change.

Acid hydrolysis of drug substance and drug product in solution state was conducted with 0.5N hydrochloric acid for 48 h. During the initial forced degradation experiments it was observed that basic hydrolysis was a fast reaction for both the drugs and almost complete degradation of both drugs occurred when 0.5N sodium hydroxide solution. Thus, in later experiment, base hydrolysis of drug substance and drug product in solution state was conducted by 0.1N sodium hydroxide solution for 2 h. For oxidative stress, sample solutions of drug substance and drug product in 3% hydrogen peroxide were kept at ambient temperature for 48 h. AM was also subjected to oxidative degradation, as mentioned in the EP, [5] to generate 'impurity D'.

3. Results and discussion

The reported method for simultaneous determination of AM and BH in combination product [13], does not give data on specificity for their estimation in the presence of degradants or impurities. This method describes a reversed-phase HPLC procedure employing a C18 column and mobile phase comprising 0.00134 M EDTA–acetonitrile–acetic acid (50:50:0.1, v/v). The EP 2002 monograph for AB [5] also states a reversed-phase HPLC method using C18 column for its assay and related compounds. The mobile phase composition in this method is buffer–acetonitrile–methanol (50:15:35, v/v) with pH of buffer adjusted to 3.0. Using these methods it was not possible to separate AM and BH and the degradation products generated during forced degradation studies. To develop the stability indicating method, first the retention behavior of AM and BH with change in apparent pH of mobile phase and with change in proportion of organic solvent (acetonitrile) were studied on

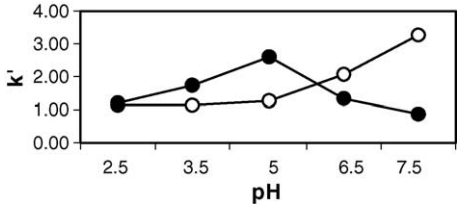


Fig. 1. Effect of change in apparent pH of mobile phase.

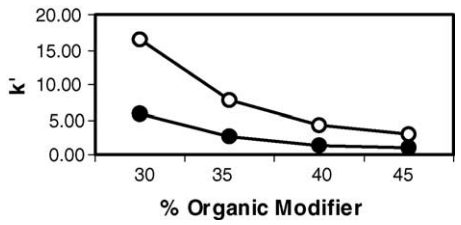


Fig. 2. Effect of change in composition of mobile phase on retention of AM (hollow circle) and BH (filled circles).

Table 1
Results of analysis of forced degradation study samples using proposed method, indicating percentage degradation of AM and BH, and purity of AM and BH peaks in chromatograms

Stress condition/duration/state	AM		BH	
	(%) Degradation	Peak purity[a]	(%) Degradationn	Peak purity[a]
Thermal/80 °C/48 h/solid	2.4	999	1.1	999
Photo/UV 254 nm/48 h/solid	0.6	999	6.0	999
Photo/UV 366 nm/48 h/solid	4.6	999	2.5	999
Thermal/80 °C/48 h/solution	12.2	999	6.2	999
Photo/UV 254 nm/48 h/solution	11.0	999	5.4	999
Photo/UV 366 nm/48 h/solution	57.4	999	30.6	999
Acidic/0.5N HCl/48 h	58.1	999	20.8	999
Alkaline/0.1N KOH/2 h	85.2	999	94.9	999
Oxidative/3.0%/48 h	72.4	999	56.6	999

[a] Peak purity values in the range 990–1000 indicate a homogenous peak.

C_{18} column. While assessing the effect of pH on the retention of analytes, the mobile phase composition was, buffer–acetonitrile (60:40, v/v); whereas assessing the effect of change of proportion of organic solvent in mobile phase, the pH of mobile was maintained at 7.0. Retention factors of both the drugs were plotted against pH of mobile phase (Fig. 1) and against proportion of organic modifier (Fig. 2), respectively.

Fig. 1 indicates that the maximum resolution between AM and BH is obtained when the pH of mobile phase is in the range of 5.0 and 7.0–7.5. When mobile phase with apparent 5.0 was used, BH is retained more than AM, and some of degradants generated during photolysis and oxidative degradation of BH were not resolved from AM. These were not resolved even with change in composition of mobile phase. But when apparent pH of mobile phase was adjusted to 7.0, AB and BH were resolved from degradants peaks at a mobile phase composition of buffer–acetonitrile (65:35, v/v). Efforts to reduce the runtime of method using the relatively less nonpolar C8 stationary phase did not succeed, as the degradants

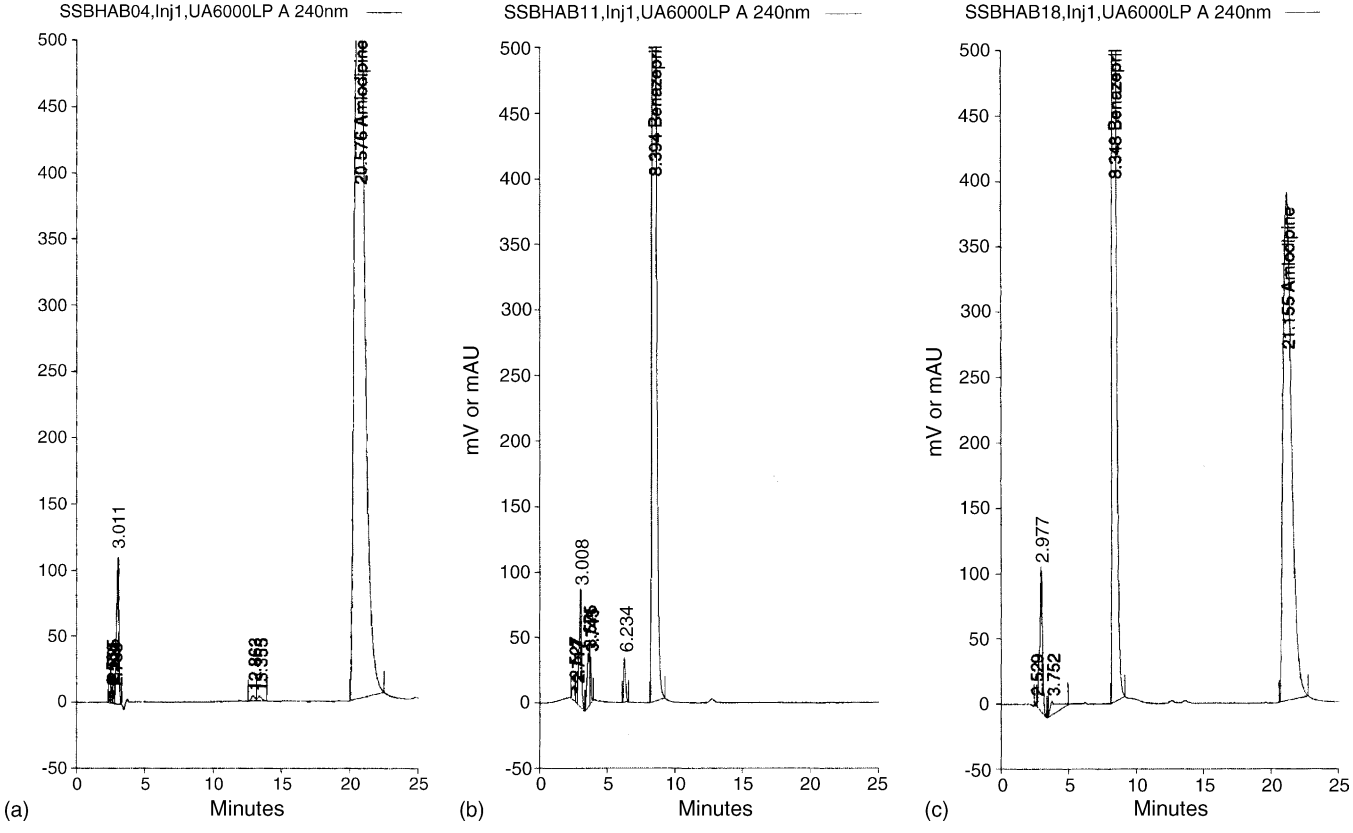


Fig. 3. (a), (b) and (c): Chromatograms of untreated AM, BH and tablet solutions, respectively.

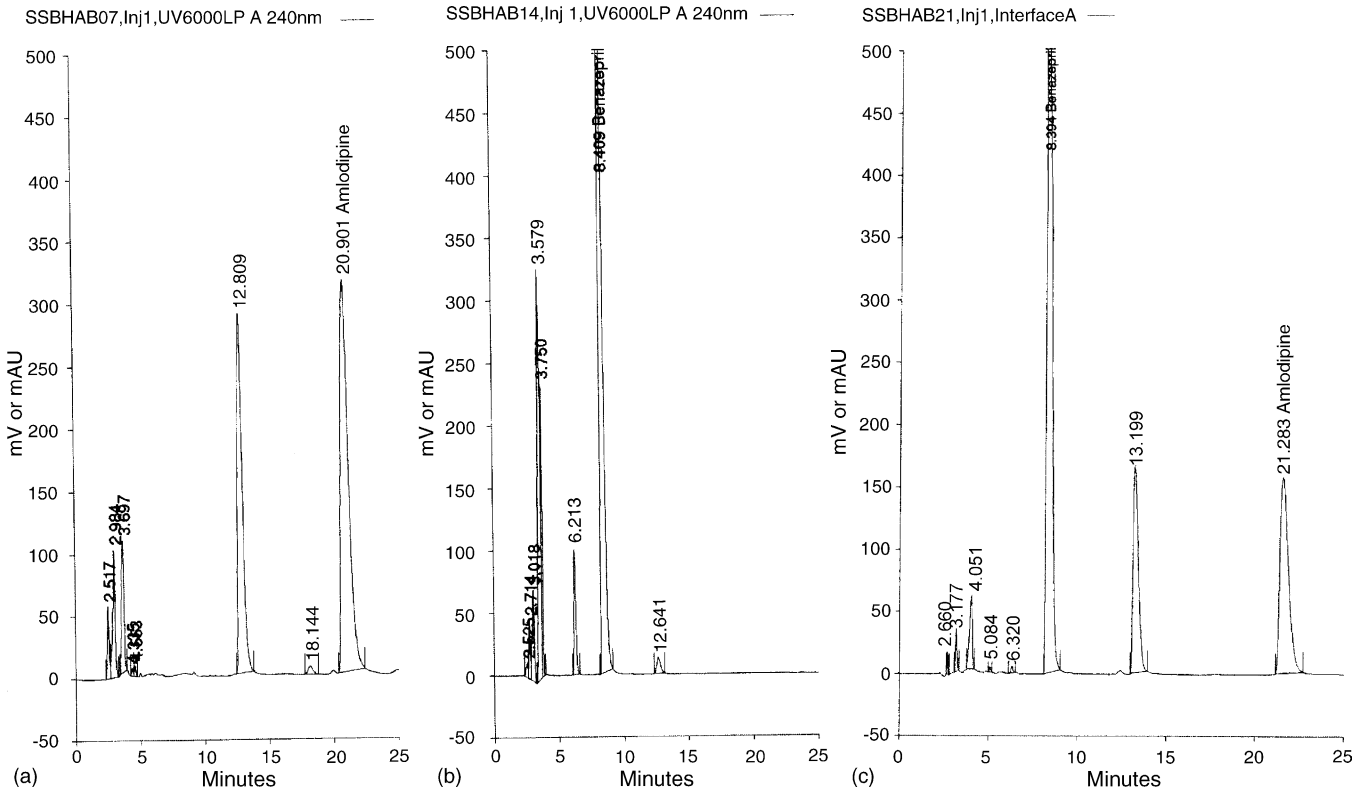


Fig. 4. (a), (b) (c): Chromatograms of photodegraded (UV 366 nm) AM, BH and tablet solutions, respectively.

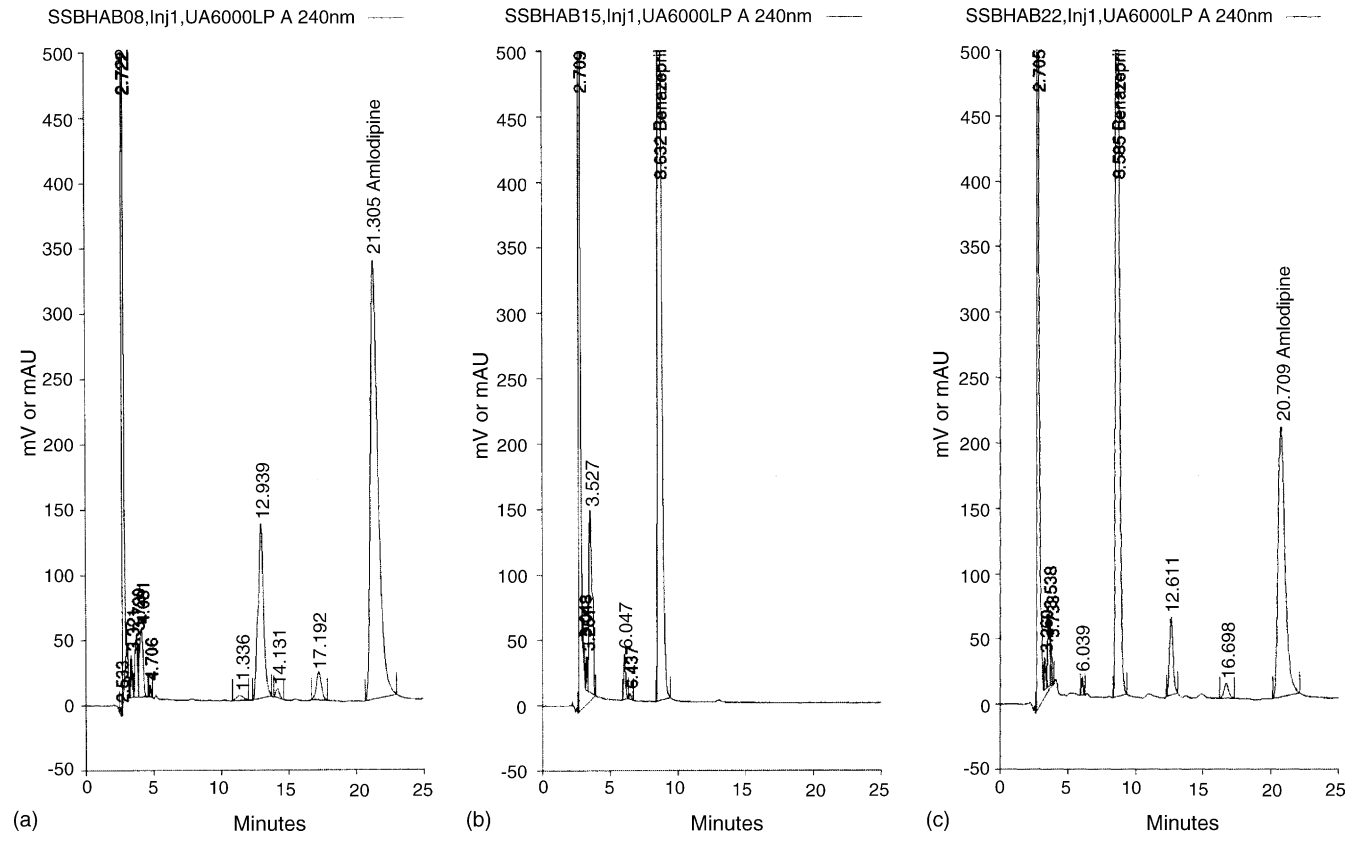


Fig. 5. (a), (b) and (c): Chromatograms of acid hydrolysis-degraded AM, BH and tablet solutions, respectively.

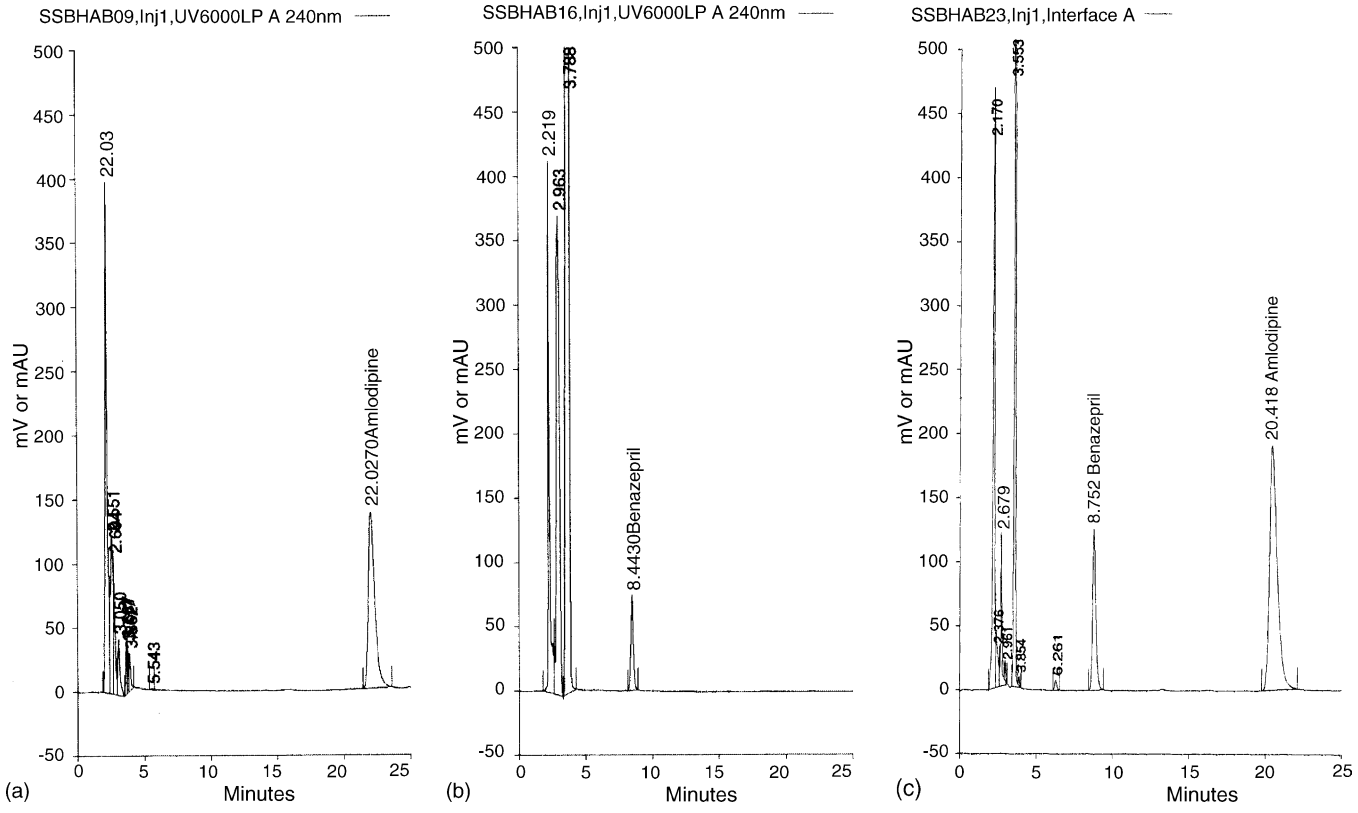


Fig. 6. (a), (b) and (c): Chromatograms of base hydrolysis-degraded AM, BH and tablet solutions, respectively.

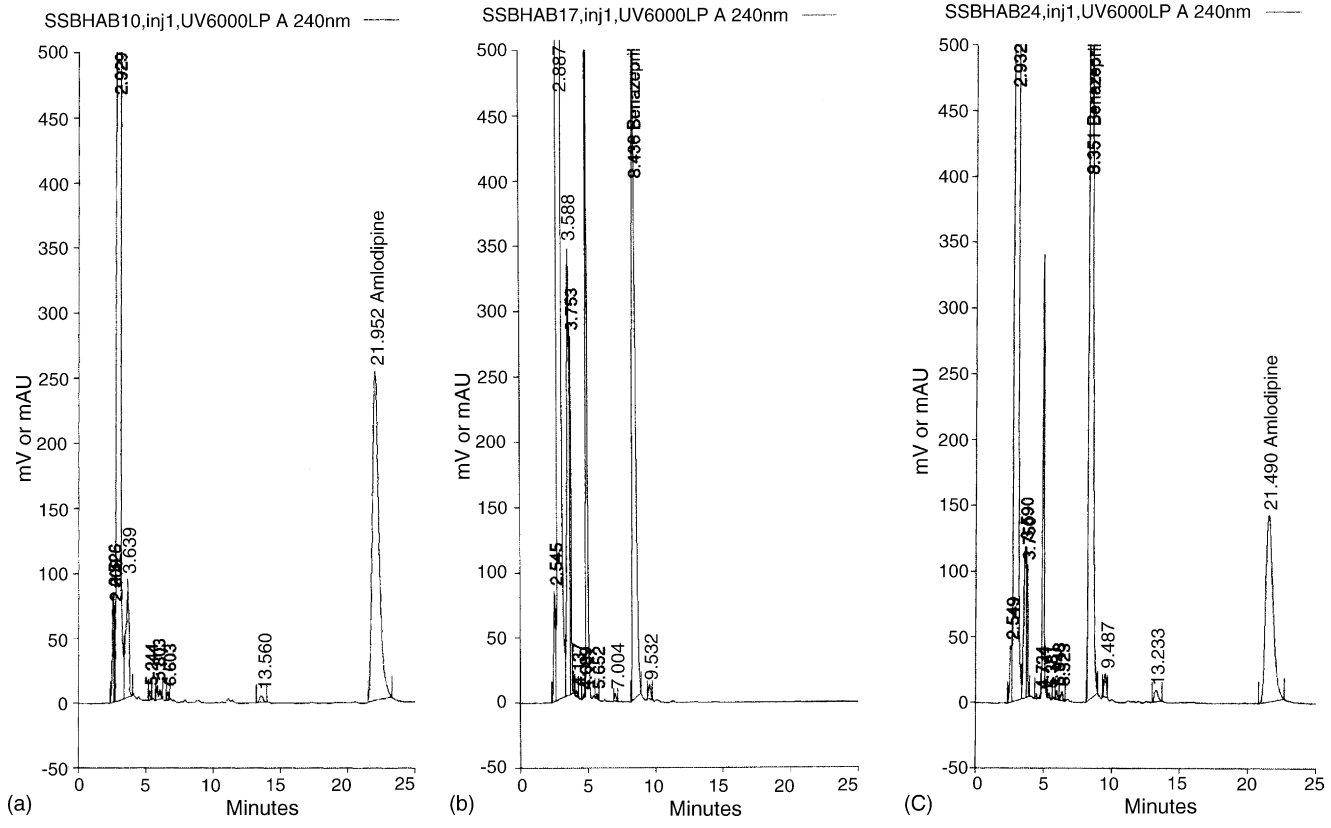


Fig. 7. (a), (b) and (c): Chromatograms of oxidative-degraded AM, BH and tablet solutions, respectively.

generated during oxidative degradation of AM interfered with the BH peak.

Singh and Bakshi, in their article on stress testing [54], suggested a target degradation of 20–80% for the establishing stability indicating nature of the assay method, as even intermediate degradation products should not interfere with any stage of drug analysis. Though condition used for forced degradation were attenuated to achieve degradation in the range of 20–80%, this could not be achieved in case of thermal and photolytic (254 nm) degradation even after exposure for prolonged duration. Significant photodegradation of AM was observed in solution state when exposed to UV radiation having peak intensity at 366 nm. Both the drugs showed extensive degradation in basic hydrolytic condition. Table 1 indicates the extent of degradation of AM and BH under various stress conditions. Photodiode array detection was used as an evidence of the specificity of the method, and to evaluate the homogeneity of the drug peak. Chromatographic peak purity data was obtained from the spectral analysis report and a peak purity value greater than 990 indicates a homogenous peak. The peak purity values for analyte peaks, AM and BH, in chromatograms of stressed samples were in the range of 999–1000 for drug substance, and in the range of 997–999 for tablets, indicating homogenous peaks and thus establishing the specificity of assay method. Resolution between the analyte peaks and nearest peak was more than 2.0 in all the chromatograms. Figs. 3–7Figs. 3(a-c) to 7(a-c) show the chromatograms of forced degraded samples. 'Impurity D' obtained by oxidative degradation of AM as mentioned in EP [5] was also well separated from both AM and BH peaks (Fig. 8).

3.1. Method validation

The described method has been validated, apart from specificity, for response function, accuracy, and intermediate precision. The nominal concentrations of standard and test solutions for AM and BH were 10 and 20 μg/ml, respectively. Response function was determined by preparing standard solution at five different concentration levels ranging form 6.0 to 14.0 μg/ml for AM and 12 to 28 μg/ml for BH. The standard solutions for linearity were prepared five times and inter-run precision for slope of regressed line was found to be 1.21 and 0.99% R.S.D. for AM and BH, respectively. The correlation coefficients were found to be more than 0.998 for both the drugs. Accuracy and precision of the method was determined by performing the recovery experiment. This experiment was performed at three levels, in which sample stock solutions were spiked with standard drug solution containing 10, 20 and 30% of labeled amount of both the drugs (5.0 mg AM and 10 mg BH) in tablets. Three replicate samples of each concentration level were prepared and the % recovery at each level ($n = 3$), and mean % recovery ($n = 9$) were determined (Table 2(b)). The mean recovery was 99.91 and 100.53% for AM and BH, respectively. A batch of tablets was analysed by two different analysts on different days using

Table 2
Method validation data for AM and BH

(a) Regression characteristics of the proposed HPLC method

Linearity experiment ($n = 5$)	AM	BH
Range (μg/ml)	6.0–14.0	12.0–28.0
Mean '*r*' value	0.9999	0.9999
Slope	139740	72632
Intercept	40163	13920
Standard error	5895.5	5787.9

(b) Results of accuracy and precision experiment using proposed method

Accuracy and precision ($n = 3$)	AM			BH		
	Taken (mg)	Recovered (mg)	(%) recovery	Taken (mg)	Recovered (mg)	(%) recovery
Level 1	0.500	0.499	99.8	0.987	0.993	100.6
Level 2	1.000	0.998	99.8	1.974	1.968	99.7
Level 3	1.500	1.502	100.1	2.961	2.999	101.3
Mean % recovery ($n = 9$)		99.9			100.5	
% R.S.D.		0.19			0.79	

(c) Results of intermediate precision for AM and BH assay in tablet formulation using proposed method

Intermediate precision	AM	BH
	Mean assay (%)/%R.S.D	Mean assay (%)/%R.S.D.
Set 1 ($n = 5$)	100.6/0.91	99.0/0.96
Set 2 ($n = 5$)	100.9/0.61	99.6/0.63
	Calculated value/critical value	Calculated value/critical value
F-test	2.212/6.388	2.353/6.388
t-test	0.600/2.306	1.198/2.306

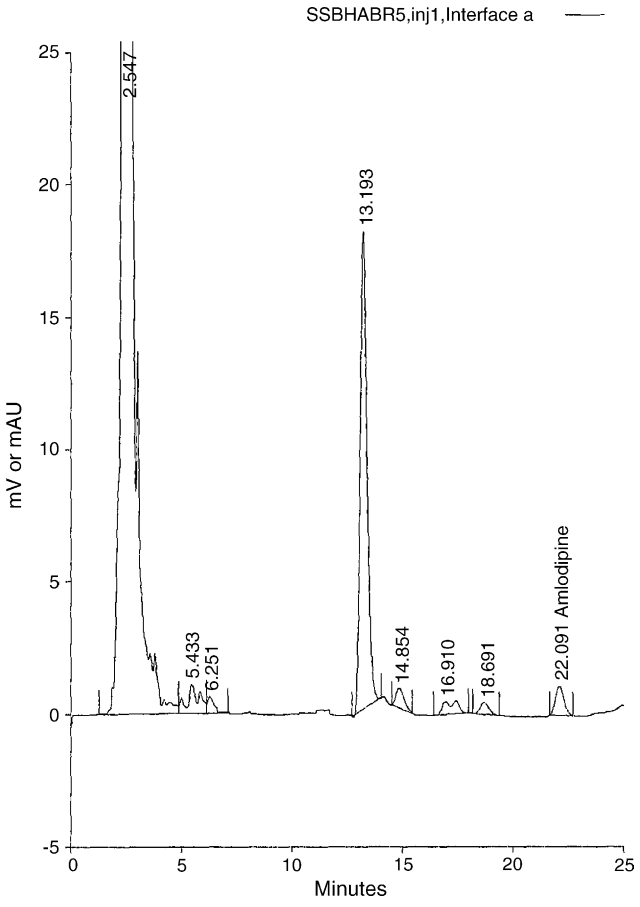


Fig. 8. Chromatogram showing oxidative degradation impurity 'D' of amlodipine at 13.19 min.

different columns to establish the intermediate precision of the method. *F*-test and *t*-test was applied to the two sets of data at 95% confidence level, and no statistically significant difference was observed.

Based on the peak purity results, obtained from the analysis of forced degraded samples using the described method, it can be concluded that the method is specific for estimation of AM and BH in presence of degradants. The method has linear response in stated range and is accurate and precise. The described method can be used as stability indicating method for assay of AM and BH in their combination product.

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