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Research Article

DEVELOPMENT AND VALIDATION OF RPHPLC METHOD FOR SIMULTANEOUS ESTIMATION OF AMLODIPINE BESYLATE AND BENAZEPRIL HCL IN TABLET DOSAGE FORM

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ABSTRACT

A simple, selective and rapid reversed phase high performance liquid chromatographic (RP-HPLC) method for the analysis of Benzapril and Amlodipine in tablet has been developed and validated. The chromatographic separation was achieved on (Inertsil ODS, 250 x 4.6mm, 5µ column) analytical column. The separation was achieved from C18 column at ambient temperature with a mobile phase consisting of methanol-acetonitrile buffer (solution of ammonium acetate and sodium pentanesulphonate ratio, 55:10:35 v/v, PH=3.00 adjusted with phosphoric acid) at a flow rate of 1ml/min and the retention time was about 1.67 minutes for benzapril and 5.0 minutes for amlodipine. The method is selective and able to resolve drug peaks from formulation excipients. The peaks of benzapril and amlodipine were well separated (resolution 11.65). The calibration curves were linear over the concentration range of 80% to 120% (r2 = 0.999 for both the drugs). The proposed method is accurate with 100.72% recovery for benzapril and 99.44% recovery for amlodipine and precise (%RSD of intraday variation were 0.53-0.152 for benzapril and 0.067-1.518 for amlodipine and %RSD of inter day variation were 0.024-1.518 for benzapril and 0.034-1.518 for amlodipine). The method has been used to test market products (six brands) and potency was found within limit (99.02%- 100.02% for benzapril and 97.4%-100.4% for amlodipine). Therefore, this method can be used as a more convenient and efficient option for the analysis of benzapril and amlodipine in tablet dosage form.

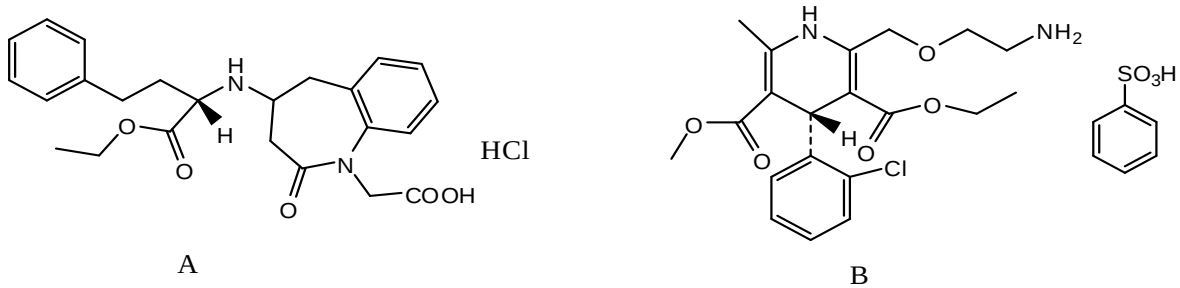
Keywords: Benzapril, Amlodipine, Method validation, HPLC, Quantitative analysis.

INTRODUCTION

Benazepril hydrochloride (Figure 1) [BEN; (3S)-3-[(1S)-1-ethoxycarbonyl -3-phenylpropylamino]-2, 3, 4, 5-tetrahydro-2-oxo-1H-1-benzazepin-1-yl] acetic acid hydrochloride is an antihypertensive drug, which belongs to the group of angiotensin convertase inhibitors. It acts on the renin-angiotensin-aldosterone system by enhibition of the conversion of the inactive angiotensin I to the highly potent vasoconstrictor - angiotensin II. It also reduces the degradation of bradykinin. BEN is applied in pharmacotherapy as a first choice drug for treatment of: arterial hypertension, ischemic heart disease, hypertrophy of the left heart ventricle and postinfarctial heart dysfunction [1-4]. Several analytical methods have been reported for the quantitative determination of BEN such as VIS-spectrophotometry [5], high performance liquid chromatography (HPLC) with ultraviolet (UV) detection [6-9], gas chromatography/mass spectrometry (GC/MS) for BEN in human plasma [10] and HPTLC-densitometry for the simultaneous determination of benazepril hydrochloride and hydrochlorothiazide in their binary mixtures. Nevertheless, no official method for resolution and determination of impurities in BEN substance has been described in the European Pharmacopoeia or British Pharmacopoeia. Moreover, some of the methods mentioned above require expensive apparatus (eg. HPLC-GC) and a long sample assay (> 15 min). According to ICH requirements, methods for kinetic studies must be not only selective, precise and accurate but also fast and unexpensive.

Amlodipine besylate (Figure 1) is a long-acting calcium channel blocker (dihydropyridine derivative) used as an anti-hypertensive and in the treatment of angina [11]. Like other calcium channel blockers, amlodipine acts by relaxing the smooth muscle in the arterial wall, decreasing total peripheral resistance and hence reducing blood pressure; in angina it increases blood flow to the heart muscle [12]. Few bioanalytical methods by HPLC [13-14] and GC [15] using human plasma have been reported for the assay of Amlodipine besylate. It is an official drug in IP [16] and BP [17]. Benazepril Hydrochloride is a non-sulfhydryl angiotensin-converting enzyme (ACE) inhibitor [18] used to treat high blood pressure (hypertension), congestive heart failure and chronic renal failure [19]. There are very few reports on analytical methods for the estimation of Benazepril HCl in human plasma [20].

The combination of Amlodipine besylate and Benazepril HCl is very useful in the treatment of hypertension. On literature survey, it was found that only bioanalytical [21] and RP-HPLC [22-24] methods have been reported for the simultaneous estimation of Amlodipine besylate and Benazepril in combined dosage forms and no method is available in the pharmacopoeias. In view of the need for suitable methods for routine analysis in combined formulations, attempts are being made to develop simple, precise and accurate analytical methods for simultaneous estimation of titled drugs and extend it for their determination in formulations. As chromatographic methods of analysis is a pre-requisite for the marketing of most of the formulations, one HPLC and HPTLC along with Spectrophotometric methods such as simultaneous equation method (Vierodt's method), first order derivative method, area under the curve method and Q-ratio method are planned to develop and validate for the simultaneous estimation of titled drugs, using water as solvent.



A B

Fig. 1: Chemical structures of (A) Benzapril and (B) Amlodipine besylate

MATERIALS AND METHODS

Material

The amlodipine besylate and benzapril were obtained from Biocon Limited, Bangalore, India and Troikaa Pharmaceuticals Ltd. respectively as gift samples. Acetonitrile (HPLC Grade) and Methanol (HPLC Grade) were purchased from E. Merck (India) Ltd. Worli, Mumbai, India. Ammonium acetate, sodium pentanesulphonate, orthophosphoric acid and other reagents were of analytical-reagent grade and purchased from E. Merck, Darmstadt, Germany. The 0.45-µm nylon filters were purchased from Advanced Micro Devices Pvt. Ltd. Chandigarh, India. Mili-Q water was used throughout the experiment.

Equipments

Analysis was performed on a chromatographic system Agilent 1200 series separation module (Japan) equipped with an auto injector (G1329A), Diode array detector (DAD) SL (G1315C), Quaternary pump (G1311A) and column thermostat (G1316A). A chromatographic separation was achieved by Symmetry C-18, 250 x 4.6mm, 5µ analytical column. Data acquisition was made with Chemstation software. The peak purity was checked with the DAD detector.

Chromatographic conditions

The mobile phase, a mixture of buffer, methanol and acetonitrile (35:55:10 v/v) pumped at a flow rate of 1.0 ml/min through the column (Inertsil ODS, 250 x 4.6mm, 5µ column) at ambient temperature. The mobile phase was degassed prior to use under vacuum by filtration through a 0.2µ nylon membrane. Concentrations were measured at 237 nm by UV detector at a sensitivity of 0.0001.

Preparation of standard solution

For 100% standard solution of target concentration 50 mg benzapril and 5 mg amlodipine (as amlodipine besylate) were weighed, dissolved and sonicated in mobile phase to produce 100 ml. 80%, 90%, 110%, and 120% standard solutions of target concentration were also prepared in the same way.

Preparation of sample solution

20 tablets were accurately weighed and the average weight was calculated. The tablets were grinded to a fine powder with the help of mortar and pestle. Then, the amount of powder equivalent to average weight of a tablet was transferred to a volumetric flask, dissolved in mobile phase and shaken for about 10 minutes then filtered through filter paper. The filtered solution was further diluted in the mobile phase to make the final concentration of working sample equivalent to 100% of target concentration.

Development and validation of HPLC Method

Present study was conducted to obtain a new, affordable, cost-effective and convenient method for HPLC determination of benzapril and amlodipine in tablet dosage form. The experiment was carried out according to the official specifications of USP–30, ICH-1996, and Global Quality Guidelines-2002. The method was validated for the parameters like system suitability, selectivity, linearity, accuracy, precision, and robustness.

System suitability

The system suitability was assessed by six replicate analysis of benzapril and amlodipine at a 100% level to verify the resolution and reproducibility of the chromatographic system adequate for the analysis to be done. This method was evaluated by analyzing the repeatability of retention time, peak area for both benzapril and amlodipine tailing factor, theoretical plates (Tangent) of the column and resolution between the peaks of benzapril and amlodipine.

Selectivity

Selectivity was determined in the presence of common excipients used in the tablet formulation. Sample containing 100% benzapril and amlodipine was injected first. Then the samples mixed with three different placebo formulations were injected to find out the selectivity of the method

Linearity

Linearity of the method was determined by constructing calibration curves. Standard solutions of benzapril and amlodipine at different concentrations level (80%, 90%, 100%, 110%, and 120%) were used for this purpose. Before injection of the solutions, the column was equilibrated for at least 30 min with the mobile phase. Each measurement was carried out in six replicates to verify the reproducibility of the detector response at each concentration level. The peak areas of the chromatograms were plotted against the concentrations of benzapril and amlodipine to obtain the calibration curves. The five concentrations of the standard were subjected to regression analysis to calculate calibration equation and correlation coefficients.

Accuracy

The accuracy is the closeness of agreement between the true value and test result. Accuracy was determined by means of recovery experiments, by addition of active drug to placebo formulations. The accuracy was calculated from the test results as the percentage of the analyte recovered by the assay.

Precision

The precision of the method was determined by repeatability (intra-day) and intermediate precision (inter-day) study. Repeatability was determined by performing four repeated analysis of the three standard solutions (90%, 100% and 110% of target concentration) of benzapril and amlodipine on the same day, under the same experimental conditions. The intermediate precision of the method was assessed by carrying out the analysis of previous standard solutions on three different days (inter-day) in the same laboratory. The relative standard deviation (% RSD) was determined in order to assess the precision of the method.

Robustness

The robustness of the method was assessed by altering the some experimental conditions such as, by changing the flow rate from 0.9 to1.1 ml/min, amount of acetonitrile (10% to 15%,) the temperature of the column (28 °C to 32 °C) and PH of the mobile phase.

RESULTS AND DISCUSSION

System suitability

The system suitability tests were carried out to evaluate the resolution and reproducibility of the system for the analysis. Table 1 summarized the test results of system suitability study. All the chromatograms showed the same retention time for benzapril (1.673 min with % RSD 0.084) and amlodipine (5.025 min with %RSD 0.39) from the six consecutive injections of the standard solution which indicates a good system for analysis. The mean theoretical plate count, based on USP tangent calculations was 3041.63 for benzapril and 2984.31for amlodipine and the resolution between benzapril and amlodipine was 11.59 Table 1. Result of system suitability tests of benzapril and amlodipine.

Selectivity

Selectivity is the ability to assess the analyte in the presence of components that may be expected to be present. Typically these might include impurities, degraded products, matrix, etc. Standard solutions (100%) containing both the drugs was injected first. Then drugs solution containing three placebo formulations were injected one after another. Figure 2 showed that in the presence of excipients the retention time of benzapril and Amlodipine remain same. On the other hand no other peaks were found within 10 min run time of the chromatogram which proves the selectivity of the method.

Linearity

Linearity of the method was evaluated from the correlation coefficient of calibration curves that were constructed from average

peak area of benzapril and amlodipine at different concentrations level (80%, 90%, 100%, 110%, and 120%). Correlation coefficient was 0.999 both for benzapril and amlodipine which prove that the method is linear for both benzapril and amlodipine. It means that the response is directly proportional to the concentration of analytes.

Accuracy

Accuracy is generally assessed by analyzing a sample with known concentration and comparing the measured value with the true value. The measured value was obtained by recovery test. Spiked amount of both benzapril and amlodipine were plotted against the recovery amount (Figure 3 and 4). The Correlation coefficient was 0.999 both for benzapril and amlodipine. In case of benzapril % recovery was 99.51% - 101.34% (average 100.72, % RSD 1.03) and in case of amlodipine % recovery was 98.86% -100.14% (average 99.44 % RSD 0.648). All the results indicate that the method is highly accurate.

Table 1: System suitability of benzapril and amlodipine

For Benzapril

Injection No.	Retention time Area	Area	Theoretical plates	Tailing factor
1	1.673	3676546.00	3048.025	1.406
2	1.671	3667323.00	3027.843	1.405
3	1.675	3675214.00	3018.468	1.406
4	1.677	3645944.00	3069.15	1.406
5	1.672	3616674.00	3047.843	1.405
6	1.673	3627404.00	3038.468	1.406
Average	1.673	3651518.00	3041.63	1.405
SD	0.001	835.228	0.504	0.001
%RSD	0.084	0.023	0.016	0.037

For Amlodipine

Injection No.	Retention time Area	Area	Theoretical Plates	Tailing factor	Resolution
1	5.025	1353496.00	2982.438	1.402	11.65
2	5.017	1354432.00	2978.862	1.401	11.71
3	5.004	1354197.00	2976.722	1.403	11.62
4	4.996	1353220.00	2983.002	1.406	11.51
5	4.973	1352243.00	2989.282	1.402	11.61
6	4.992	1351266.00	2995.562	1.406	11.49
Average	5.001	1353142.33	2984.311	1.407	11.59
SD	0.023	1203.005	6.983	0.002	0.046
%RSD	0.390	0.089	0.234	0.162	0.405

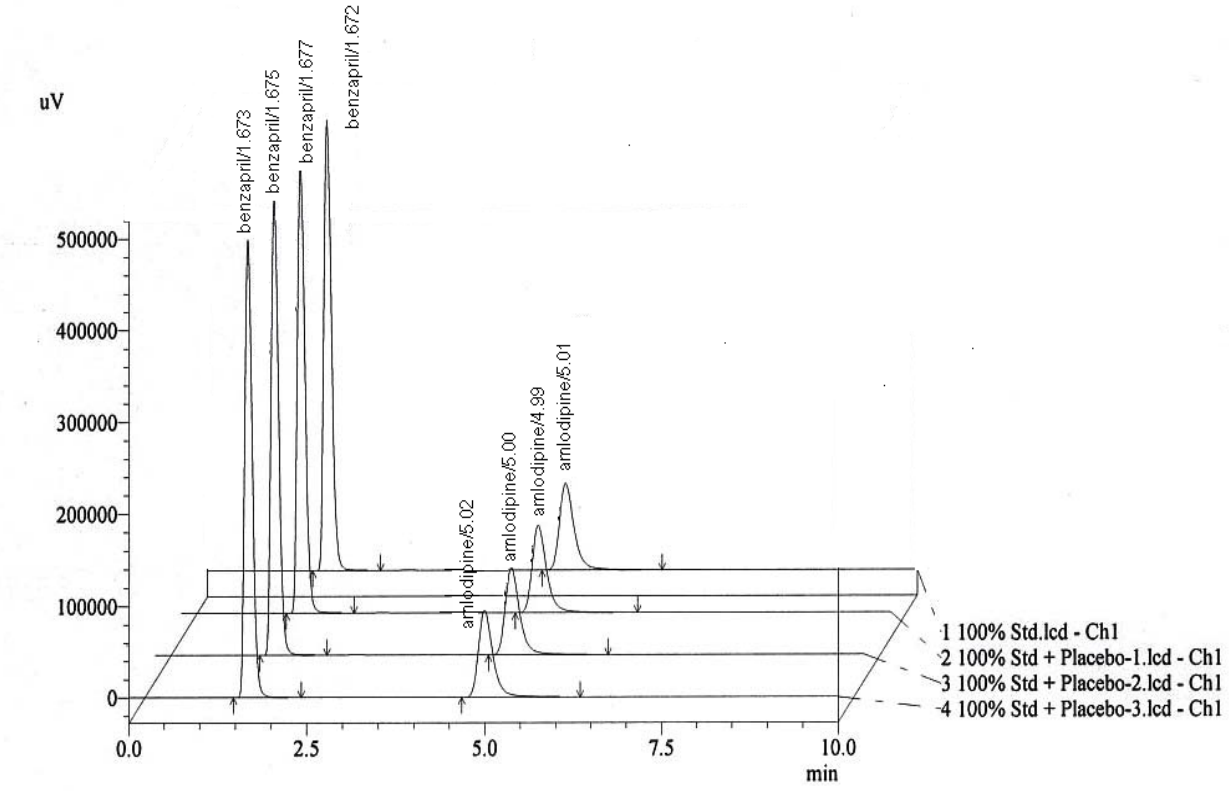


Fig. 2: Chromatogram of benzapril and amlodipine along with 3 placebo formulations.

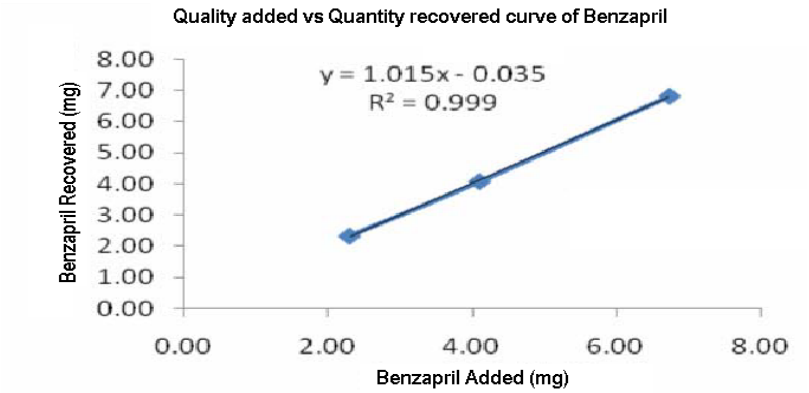


Fig. 3: Accuracy curve of benzapril

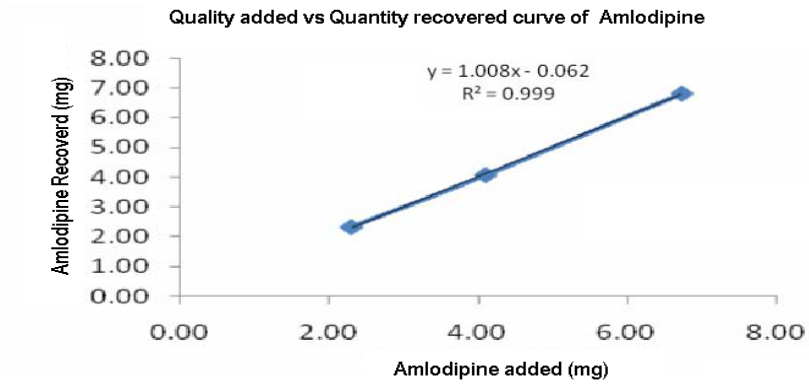


Fig. 4: Accuracy curve of amlodipine

Precision

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple samplings of a homogeneous mixture. The precision of an analytical method is usually expressed as the standard deviation or relative standard deviation (coefficient of variation) of a series of measurements. Precision was measured by repeatability and intermediate precision.

Repeatability

Repeatability expresses the precision under the same operating conditions over a short interval of time. It is also termed intra-assay precision. Repeatability is usually demonstrated by repeated measurements of a single sample. Minimum four determinations at each of three concentrations across the intended range, or a minimum of six determinations at the test concentration are recommended. The measurements for repeatability were done from 9.00 am to 9.00 pm. Four determinations of three concentrations across the intended range (90%, 100% and 110% of target concentration) were included in the study. % RSD of peak areas was calculated for various run. The method is highly precise as % RSD of peak area was 0.053-0.152 in case of benzapril and 0.076-1.518 in case of amlodipine.

Intermediate precision

Intermediate precision is usually demonstrated by repeated measurements of the sample used in the repeatability experiment within the same laboratory. Usually the repeatability experiment is repeated on the same sample by a different analyst, on a different day, using different equipment if possible. The data of table 2 and 3 showed the average results of intermediate precision of benzapril and amlodipine. The same concentration levels as in the repeatability experiment were used in this study. The results are obtained by 3 concentrations with 4 runs over 3 days. The average peak area obtained at different levels and different days indicate that the method is precise. Maximum % RSD is 0.151 in case of benzapril and 1.51 in case of amlodipine.

Table 2: Inter-day variability (three different concentrations of standard solution of benzapril, injected on different days)

Days	Std conc.	Mean peak area of benzapril (n=4)	% RSD
	90%	3311515.25	0.0797
1st	100%	3686255.75	0.0243
	110%	4031586.50	0.1105
	90%	3312999.75	0.0529
2nd	100%	3686025.25	0.1518
	110%	4033851.00	0.0854
	90%	3332855.75	0.0767
3rd	100%	3706295.75	0.0556
	110%	4069579.75	0.069

Table 3: Inter-day variability (three different concentrations of standard solution of amlodipine injected on different days)

Days	Std conc.	Mean peak area of amlodipine (n=4)	% RSD
	90%	1299744.00	0.1667
1st	100%	1353835.75	0.0422
	110%	1613112.00	0.1691
	90%	1300537.00	0.076
2nd	100%	1377701.25	1.5183
	110%	1616511.00	0.2024
	90%	1315026.75	0.1273
3rd	100%	1368271.75	0.0341
	110%	1633389.75	0.1308

Table 4: Results for robustness test of benzapril and amlodipine

Parameters	Changes	% Recovery of Benzapril	% Recovery of amlodipine	% Target
Flow Rate	0.9	99.7	99.3	100
(ml/min)	1.1	99.6	99.4	100
(0C)	30	99.5	99.5	100
	10%	99.6	99.7	100
Acetonitrile Variation	15%	99.6	99.6	100

Robustness

The robustness of a method is its ability to remain unaffected by small changes. Robustness study was performed by making slight variations in flow rate, amount of acetonitrile, and temperature. 100% benzapril and amlodipine sample solution was used in this study. The results of robustness in the present method showed no significant changes occurring over changes which are summarized in Table 4. As the changes are not significant we can say that the method is robust.

CONCLUSION

The proposed high-performance liquid chromatographic method has been evaluated over the accuracy, precision and linearity and proved to be more convenient and effective for the quality control and identity of benzapril and amlodipine in pharmaceutical dosage forms. The measured signals were shown to be precise, accurate and linear over the concentration range tested (80–120% of target concentration) with a correlation coefficient better than 0.999. Moreover, the lower solvent consumption along with the short analytical run time of 10 minutes leads to an environmentally friendly chromatographic procedure that allows the analysis of a large number of samples in a short period of time. Therefore, this HPLC method can be used as a routine sample analysis. Additionally in this method, there was no interference from matrix sources.

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