ORIGINAL ARTICLE

SIMULTANEOUS HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF ATENOLOL AND AMLODIPINE IN PHARMACEUTICAL-DOSAGE FORM

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ABSTRACT

A simple, rapid and precise method is developed for the quantitative simultaneous determination of atenolol and amlodipine in a combined pharmaceutical-dosage form. The method is based on High Performance Liquid Chromatography (HPLC) on a reversed-phase column, shim-pack CLC, ODS (C18), 4.6 mm × 25 cm & 0.5 μm, using a mobile phase of ammonium acetate buffer (the pH was adjusted to 4.5 ± 0.05 with glacial acetic acid), acetonitrile and methanol (35:30:35 v/v). The buffer used in the mobile phase contains ammonium acetate in double-distilled water. The chromatographic conditions are- flow rate of 1.5ml/min, column temperature at 40˚C and detector wavelength of 237 nm. Both the drugs were well resolved on the stationary phase and the retention times were around 1.5 minute for atenolol and 3.4 minute for amlodipine. The method was validated and shown to be linear for atenolol and amlodipine. The correlation coefficients for atenolol and amlodipine are 0.999963 and 0.999979, respectively. The relative standard deviations for six replicate measurements in two sets of each drug in the tablets is always less than 2% and mean % error of active recovery not more than ± 1.5%. The method was validated for precision and accuracy. The proposed method was successfully applied to the pharmaceutical dosage forms containing the above-mentioned drug combination without any interference by the excipients.

Keywords: Atenolol and amlodipine; validation; HPLC; tablets.

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 (Prisant, 2002). These potential advantages make it recommendable for the combination anti-hypertensive therapy to be used as initial treatment, particularly in patients with target-organ damage or more severe initial hypertension (Moser, 1998; Moser and Black, 1998). Calcium antagonists are vasodilatory and tend to increase plasma renin, therefore combination with a *β*-blocker is theoretically sound (Waeber *et al.,* 1999).

Amlodipine, with its intrinsically long half-life alone or together with *β*-blocker, is likely to produce superior ischaemia reduction in clinical practice when patients frequently forget to take medication or take doses irregularly (Deanfield *et al.,* 2002; Davies *et al.,* 1995). Mettimano *et al*. (2000) found that adding amlodipine to atenolol produced a significant reduction in blood pressure when compared with placebo in patients whose blood pressure was not controlled by atenolol alone. The reduction of side-effects, obtained by adding a dihydropyridine derivate to a *β*-blocker, confirms the effectiveness of this combination (Mettimano *et al.,* 2000). It is clearly demonstrated that the combination of atenolol and amlodipine is synergistic in lowering and stabilizing BP and this synergism is highest when the dose proportion of the two drugs is 10 : 1 (Li-Ping *et al.,* 2005).

The Indian Pharmacopoeia describes non-aqueous titration method for the assay of atenolol. The British Pharmacopoeia examines amlodipine besylate by liquid chromatography. An UV-spectrophotometric (Kasture and Ramteke, 2006) and reversed phase HPLC (Ravishankar *et al*., 1997) methods are reported for simultaneous estimation of atenolol and amlodipine besylate in combined dosage form, but to the best of our knowledge, no report related to the determination of atenolol and amlodipine besylate in pharmaceutical dosage forms using a reversed-phase column, shim-pack CLC, ODS (C18), 4.6 mm × 25 cm & 0.5 μm, using a mobile phase of ammonium acetate buffer acetonitrile and methanol (35:30:35 v/v), has, so for, been mentioned in literature or in pharmacopoeias and hence the present work was undertaken.

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The focus of the present study was to develop and validate a economic, rapid reversed-phase high-performance liquid chromatographic method for the quality control of atenolol and amlodipine besylate in pharmaceutical preparations with lower solvent consumption along with the short analytical run time leads to an environmentally friendly chromatographic procedure that allows the analysis of a large number of samples in a short period of time. The proposed method is applicable as well as for routine analysis and content uniformity test of atenolol and amlodipine besylate in tablets and complies well with the validation requirements in the pharmaceutical industry.

EXPERIMENTAL

Equipment and chromatographic conditions

A high-performance liquid chromatographic system consisted of a pump LC-10 AS, oven CTO-10A, detector SPD-10A and recorder C-R6A (Shimadzu, Japan). All pH measurements were performed on a pH meter (Sentron, Netherlands).

Chromatographic separation was carried out at room temperature with shim-pack CLC, ODS (C18), 4.6 mm × 25 cm & 0.5 µm column from Shimadzu, Japan. For the mobile phase, 1.54 gm of ammonium acetate was dissolved in 900 ml of double-distilled water. The buffer solution was shaked manually to dissolve and finally make the volume up to 1000 ml with the same. A mixture of ammonium acetate buffer acetonitrile and methanol in the ratio of 35: 30: 35 was prepared. The pH of the ammonium acetate buffer was adjusted to 4.5 ± 0.05 with glacial acetic acid.

Finally the mobile phase was filtered through a 0.45 µm membrane filter and degassed for 10 minutes. The injection volumes for samples and standards were 20 µl and eluted at a flow rate of 1.5 ml/min at 40 ˚C. The eluents were monitored at 237 nm.

Materials and reagents

Acetonitrile and methanol were of HPLC grade and were purchased from E. Merck, Darmstadt, Germany. Ammonium acetate, glacial acetic acid and other reagents were of analytical-reagent grade and purchased from E. Merck, Darmstadt, Germany. Water was deionised and double distilled. Camlodin Plus tablets were kind gift from Square Pharmaceuticals Ltd Bangladesh. Each tablet was labeled to contain 50 mg atenolol and 5 mg amlodipine. The excipients present in the tablets are: microcrystalline cellulose, maize starch, sodium starch glycolate, pigments blend-24843 (pink), colloidal silicon dioxide, magnesium stearate and purified talc.

Preparation of standard solutions

A working standard solution containing atenolol 25 mg/100ml and amlodipine 2.5 mg/100ml was prepared by dissolving atenolol and amlodipine besylate reference standard in mobile phase. The mixture was sonicated for 5 minutes or until the reference standard dissolved completely.

Preparation of sample solutions

Twenty tablets, each containing 50 mg atenolol and 5 mg amlodipine were accurately weighed and finely powdered. A quantity of powder equivalent to 25 mg of atenolol and 2.5 mg of amlodipine was weighted and transferred to a 100 ml volumetric flask. About 70 ml of mobile phase was added and shaked mechanically for 15 minutes. The mixture was then sonicated in ultrasonic bath for 5 minutes and makes the volume up to 100 ml by the mobile phase. The solution was filtered with a Whatman filter paper no.1.

Before injection, both standard and sample solution was filtered through 0.45 µm syringe filter.

Then 20 µl of standard and sample solutions were injected into column and chromatogram was recorded (fig. 1).

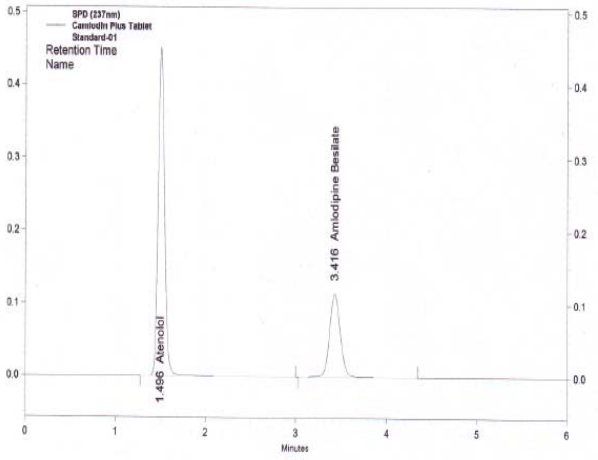


Fig. 1: HPLC chromatogram of Camlodin Plus tablet.

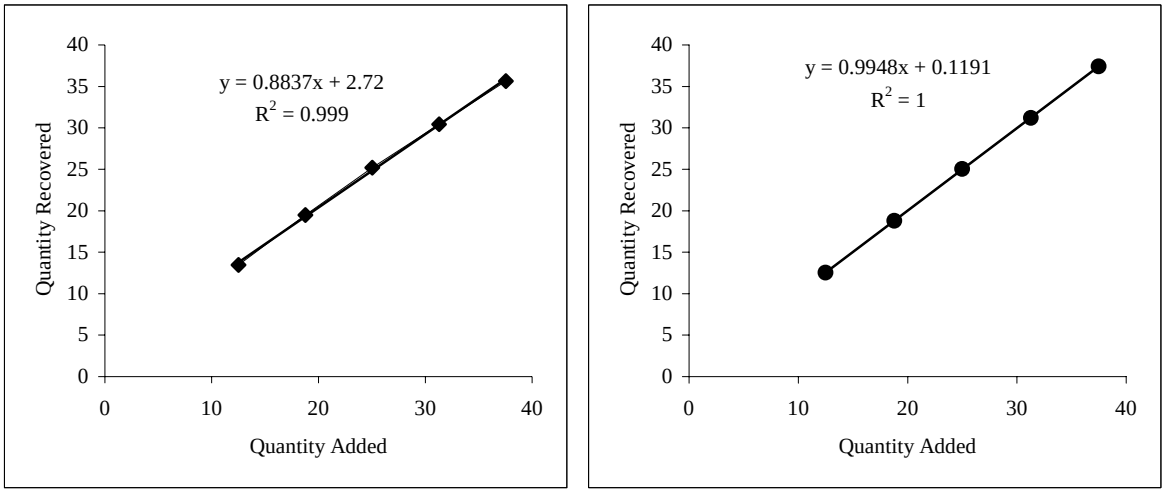
Method validation

The linearity of the analytical method is its ability to elicit test results that are directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in samples within a given range. The linearity is important to demonstrate that the response of the measurement of detector system is linear over the range of interest of the method. This was determined by means of calibration graph using increasing amounts of a standard solution (0, 50, 75, 100, 125 and 150%) of both atenolol and amlodipine.

These standards were tested six times in agreement to the International Conference on Harmonization (ICH). A

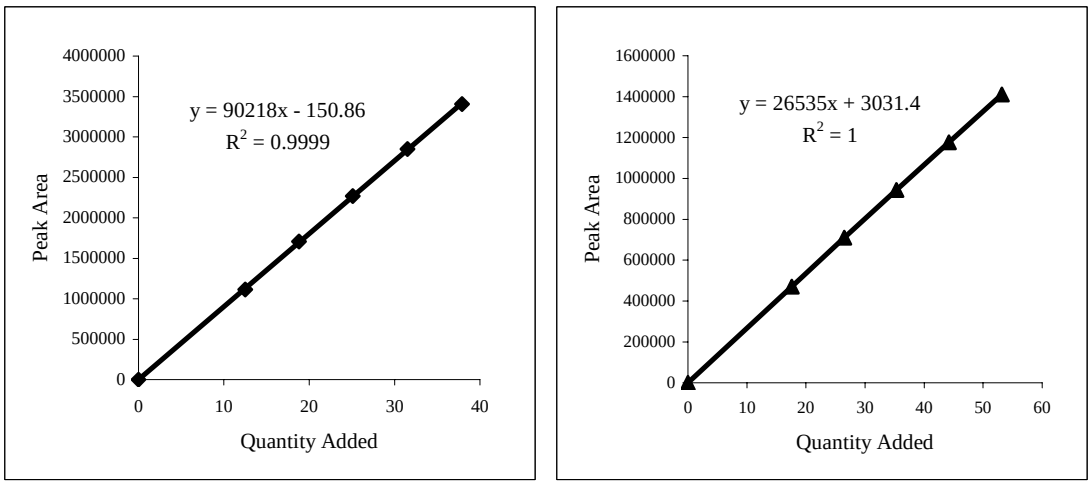
calibration curve was constructed and the proposed method was evaluated by its correlation coefficient and intercept value, calculated in the corresponding statistical study (ANOVA) ($p < 0.05$). Characteristic parameters for regression equation ($y = a + bx$) of the HPLC method obtained by least squares treatment of the results was used to confirm the good linearity of the method developed. The correlation co-efficient between concentration and Peak Area found must not be less than 0.995.

The accuracy of the assay was measured by analyzing five spiked samples of atenolol and amlodipine (50, 75, 100, 125, 150%). The accuracy of this method was the closeness of the test results obtained by that method to the true value and established across its range. Accuracy was determined by means of recovery experiments, by spiked addition of active drug to placebo formulations. It was shown that the recoveries were independent of the concentration of the active over a reasonable concentration range normally 50 to 150 % of the nominal concentration. The amount recovered was plotted against the theoretical amount which produced a straight line of



(a) Atenolol (b) Amlodipine

Fig. 2: Acuracy curves[a] of Camlodin Plus tablet; (a) Atenolol (b) Amlodipine.
[a]The experiment was performed using C18 as the column of choice and ammonium acetate buffer solution (pH 4.5) mixed with acetonitrile and methanol (35:30:35) as mobile phase.



(a) Atenolol (b) Amlodipine

Fig. 3: Calibration curves[b] of Camlodin Plus tablet; (a) Atenolol (b) Amlodipine.
[b]The experiment was performed using C18 as the column of choice and ammonium acetate buffer solution (pH 4.5) mixed with acetonitrile and methanol (35:30:35) as mobile phase.

Table 1: Summary of the results of amount added *VS* amount recovered.

Sample No.	Atenolol		Amlodipine	
	Active added (in mg)	*Active recovered (in mg)	Active added (in mg)	*Active recovered (in mg)
1	12.51	13.46 ± 0.25	12.49	12.54 ± 0.12
2	18.77	19.48 ± 0.62	18.77	18.78 ± 0.06
3	25.03	25.17 ± 0.15	24.99	25.04 ± 0.24
4	31.28	30.43 ± 0.31	31.27	31.21 ± 0.13
5	37.54	35.64 ± 0.09	37.48	37.40 ± 0.36

*Values are mean ± SEM of three determinations

Table 2: Percent of active recovered from different % of sample.

	Atenolol	Amlodipine
Active Recovered from 50% Sample (in %)	107.57%	100.35%
Active Recovered from 75% Sample (in %)	103.80%	100.01%
Active Recovered from 100% Sample (in %)	100.56%	100.20%
Active Recovered from 125% Sample (in %)	97.27%	99.78%
Active Recovered from 150% Sample (in %)	94.92%	99.77%
Average mean of Recovery	100.82%	100.02%
Mean of error	0.828%	0.0279%

Table 3: Summary data of precision of the atenolol and amlodipine HPLC determination

Precision[a]								
Test		Mg/tablet						%RSD
		S1	S2	S3	S4	S5	S6	
Repeatability	Atenolol	49.28	49.27	49.40	49.20	49.45	49.42	0.20188
	Amlodipine	4.951	4.943	4.952	4.927	4.956	4.946	0.20837

[a] The experiment was performed using C18 as the column of choice and ammonium acetate buffer solution (pH 4.5) mixed with acetonitrile and methanol (35:30:35) as mobile phase.

slope one and intercept zero. The accuracy was expressed by mean percentage error which was also to be determined and it was not more than ± 1.5 %.

According to the ICH recommendations, precision must be considered at two levels, repeatability and intermediate precision. Repeatability refers to the use of the analytical procedure within a laboratory over a short period of time using the same analyst with the same equipment. On that account, a six-sample replicates were consecutively tested in the same equipment at a concentration of 100% of both atenolol and amlodipine of the regular analytical working value. The intermediate precision expresses the within-laboratory variations, was assessed by using different equipments, analysts and days to analyze three samples six times. The relative standard deviation (% RSD) was determined in order to assess the precision of the assay and it was not be more than 2.0 %.

RESULTS AND DISCUSSION

Methods development and optimization

This isocratic-mode method with UV detection was developed for the determination of the active ingredients, atenolol and amlodipine, at 100% level. Firstly, the reversed-phase column, shim-pack CLC, ODS (C18), 4.6 mm × 25 cm & 0.5 μm was tested. The system suitability studies were carried out as specified in ICH.

The mobile phase consisted of ammonium acetate buffer solution acetonitrile and methanol at various ratios (40:30:30, 35:30:35, 30:40:30 (v/v)) was tested as starting solvent. The variation at the mobile phase leads to considerable changes in the chromatographic parameters. However, the proportion buffer: acetonitrile: methanol at a ratio of 35/30/35 (v/v) yielded the best results.

Table 4: Summary of the results of amount added vs peak area.

Sample No.	Different % of sample	Atenolol		Amlodipine	
		Active added (in mg)	Peak area	Active added (in mg)	Peak area
1	0%	0	0	0	0
2	50%	12.5	1113949	17.6	470199
3	75%	18.8	1707702	26.5	709347
4	100%	25.1	2269160	35.3	943691
5	125%	31.5	2850190	44.2	1175918
6	150%	37.9	3407552	53.2	1410377
Correlation coefficient		0.999963		0.999979	

Our data showed that the variation of the pH (3.5, 4.0, 4.5) of the ammonium acetate buffer did have significant effects on the HPLC-UV chromatographic resolution. Although the retention times of atenolol and amlodipine showed a change in the pH variation (3.0–4.5), it was necessary to maintain pH value of the buffer at 4.5 for the optimum separation of the compounds, as at this pH the analyte peaks were well defined and resolved.

In order to study the effect of excipients on quantification of atenolol and amlodipine, a placebo was prepared using microcrystalline cellulose, maize starch, sodium starch glycolate, pigment blend-24843 (pink), colloidal silicon dioxide, magnesium stearate and purified talc. The results revealed no interference of the excipients.

In order to obtain a satisfactory and full detection for this new method, 3D-UV–vis spectra of standard atenolol and amlodipine solution were obtained (data not shown). Based on the highest UV absorbance for atenolol and amlodipine, 237 nm was chosen for detection of this new HPLC method at which the best detector responses for all substances were obtained.

Method validation

The acuracy was evaluated by the recovery of atenolol and amlodipine at five different levels (50, 75, 100, 125 and 150%). An accuracy curve (fig. 2) was constructed and the summary of the results and average mean of recovery data for each level of both active pharmaceutical ingredients (API) was within accepted values was shown in tables 1 and 2.

The data of table 3 showed that average results of repeatability of atenolol and amlodipine and was within the limit and R.S.D. was 0.20188 and 0.20837, respectively, which indicated a good precision.

A calibration curve (fig. 3) was constructed and the proposed method was evaluated by its correlation coefficient (0.999963 and 0.999979) (table 4). Characteristic parameters for regression equation ($y = a + bx$) of the HPLC method obtained by least squares treatment of the results confirmed the good linearity of the method developed (fig. 3).

Label claim recoveries from Camlodin plus tablets

The proposed method was evaluated in the assay of commercially available tablets containing 50 mg of atenolol and 5 mg of amlodipine. Six replicate determinations (n=6) were carried out on an accurately weighted amount of the pulverized tablets equivalent to 25 mg of atenolol and 2.5 mg of amoldipine as amoldipine besylate. The label claim found was to be 47.96-50.21 mg of Atenolol and 4.85-5.02 mg of amlodipine per tablet (table 5).

Table 5: Determination of atenolol and amlodipine in commercial formulations by high-performance liquid chromatography

Sample No.	Atenolol (mg/tablet)	Amlodipine (mg/tablet)
Sample 1	49.56 ± 0.98	4.92 ± 0.21
Sample 2	50.21 ± 1.10	4.88 ± 0.52
Sample 3	47.96 ± 1.14	5.02 ± 0.44
Sample 4	49.67 ± 0.47	4.97 ± 0.23
Sample 5	49.42 ± 0.75	4.85 ± 0.65
Sample 6	48.90 ± 0.88	4.99 ± 0.32

Results are the mean ± S.E.M. of three independent experiments.

CONCLUSIONS

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 atenolol and amlodipine in pharmaceutical dosage forms. The measured signals were shown to be precise, accurate and linear over the concentration range tested (0–150%) with a correlation coefficient better than 0.9991. Moreover, the lower solvent consumption along with the short analytical run time of 5.0 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.

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REFERENCES

Davies RF, Habibi H and Klinke WP *et al.* (1995). Effect of amlodipine, atenolol and their combination on myocardial ischemia during treadmill exercise and ambulatory monitoring. *J. Am. Coll. Cardiol.,* **25**: 619-625.

Deanfield JE, Detry JM and Sellier P *et al.* (2002). CAPE II Trial Investigators. Medical treatment of myocardial ischemia in coronary artery disease: effect of drug regime and irregular dosing in the CAPE II trial. *J. Am. Coll. Cardiol.,* **40**: 917-925.

ICH, Q2B, (1995). Validation of analytical procedure: methodology. International Conference on Harmonisation, London.

Kasture AV and Ramteke M (2006). Simultaneous UV-spectrophotometric method for the estimation of atenolol and amlodipine besylate in combined dosage form. *Indian J. Pharm. Sci.,* **68**(3): 394-396.

Li-Ping X, Fu-Ming S, He S, Chao-Yu M, Yúan-Ying J and Ding-Feng S (2005). Synergism of atenolol and amlodipine on lowering and stabilizing blood pressure in spontaneously hypertensive rat. *Acta. Pharmacologica. Sinica,* **26**(11): 1303-1308.

Menard J (1993). Critical assessment of combination therapy development. *Blood Press. Suppl.,* **1**: 5-9.

Mettimano M, Pichetti F and Fazzari L *et al.* (2000). Combination therapy with beta-adrenergic blockade and amlodipine as second line treatment in essential hypertension. *Int. J. Clin. Pract.,* **54**: 424-428.

Moser M (1998). Current recommendations for initial therapy in hypertension: are they still valid? Introduction. *Am. J. Hypertens.,* **11**: 69S-72S.

Moser M and Black HR (1998). The role of combination therapy in the treatment of hypertension. *Am. J. Hypertens.,* **11**: 73S-78S.

Prisant LM (2002). Fixed low-dose combination in first-line treatment of hypertension. *J. Hypertens.*, **20**: S11-S19.

Ravishankar S, Nanjan MJ, Vasudevan M, Shaat N and Suresh B (1997). Simultaneous estimation of atenolol and amlodipine in formulations by reversed phase-HPLC. *Indian J. Pharm. Sci.,* **59**: 171-173.

Waeber B, Detry JM and Dahlof B *et al.* (1999). Felodipine-metoprolol combination tablet: a valuable option to initiate antihypertensive therapy? *Am. J. Hypertens.,* **12**: 915-920.