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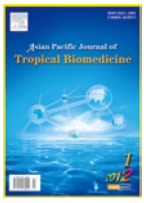

Document heading

# Development and validation of a HPLC method for the simultaneous estimation of amlodipin and telmisartan in pharmaceutical dosage form

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ABSTRACT

**Objective:** To develope and validate a simple and rapid isocratic reversed-phase high-performance liquid chromatographic method (RP-HPLC) for the simultaneous estimation of amlodipin and telmisartan in combined dosage form. **Methods:** The chromatographic separation was achieved by using mobile phase acetonitrile and 0.05M sodium dihydrogen phosphate buffer (60:40) adjusted to pH 6.0, a C-18 column, perfectsil target ODS3 (150 mm×4.6 mm *i.d.*, 5 μm). The mobile phase was pumped at a flow rate of 0.8 mL/min and the eluents were monitered at 254 nm. **Results:** Retention times were 4.0 min and 8.2 min for amlodipine and telmisartan respectively. The method was validated in terms of accuracy, precision, linearity, range, specificity, limit of detection and limit of quantitation. Linearity for amlodipine besylate and telmisartan was established in the range of 5-30 and 10-60 μg/mL, respectively. The recoveries for the two compounds were above 96%. **Conclusions:** This method was found to be efficient, accurate, precise, specific and economic and is suitable for routine quality control analyses.

## 1. Introduction

Amlodipine besylate (AMLB), a long-acting calcium channel blocker and used as an antihypertensive and an antianginal agent, which is chemically described as 3-ethyl-5-methyl(±)2- [(2 -aminoethoxy )methyl]-4-(2-chlorophenyl) 1,4-dihydro-6-methyl-3,5-pyridine dicarboxylate, monobenzenesulphonate[1,2]. Literature survey revealed HPLC[3-5], RP-HPLC[6-8], HPTLC[9,10], LCMS/MS[11], LC-MS[12] and simultaneous UV spectrophotometric methods[13,14] are reported for quantitative estimation of AMLB alone or in combination with other anti-hypertensive agents. Telmisartan (TEL), 4-{[2-n-propyl-4-methyl-6-(1-methylbenzimidazol-2-yl)-benzimidazol-1-yl] methyl}-biphenyl-2-carboxylic acid is a new highly selective, nonpeptide angiotensin II type 1 (AT1)-receptor antagonist[1]. TEL lowers blood pressure through blockade of the rennin-angiotensin-aldosterone system (RAAS) and is widely used in the treatment of hypertension[15]. Determination of TEL in human plasma by liquid chromatography-tandem mass spectrometry has been reported[16]. HPTLC[17,18], spectrophotometry and RP-HPLC method for determination of TEL in combination with other anti-hypertensive agents has been reported[19-21].

Present study involves the efficient RP-HPLC method for the estimation of AMLB and TEL in combined dosage form.

## 2. Materials and methods

TEL and AMLB standard drug was obtained as a gift sample from Lupin Limited, Pune. acetonitrile and methanol (HPLC grade), sodium dihydrogen phosphate, triethylamine (AR grade) were purchased from Merck, India. Triple distilled water was used for all purposes.

### 2.1. Instrumentation and chromatographic conditions

HPLC system (Cecil liquid chromatography) was consist of HPLC pump-cecil-CE-4100-adept series-dual piston pump, column-phenomenex (250 mm ×4.60 mm) Luna 5 μ C 18 (2) 100. Mobile phase was a mixture of acetonitrile and 0.0 5M sodium dihydrogen phosphate buffer (60:40), and was adjusted to pH 6.0 with TEA (triethyl amine). Flow rate was kept at 0.8 mL/minute and column temperature was maintained at 35 ℃, the detection was monitored at a wavelength of 254 nm and injection volume was 20 μ L.

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### 2.2. Standard solutions and calibration graphs for chromatographic measurement

Stock standard solutions of AMLB and TEL were separately prepared in methanol. 10 mg of AMLB and TEL were accurately weighed and transferred to 10 mL volumetric flask separately. They were dissolved into methanol then volume was made up with methanol (1 000 μg /mL). 1 mL of stock solution was taken and diluted to 10 mL with mobile phase to get working standard of AMLB (A) and TEL (B) (100 μg/mL). Using working aliquots of standard, solution of 5, 10, 15, 20, 25 and 30 μg/mL of AMLB and 10, 20, 30, 40, 50 and 60 μg/mL of TEL were prepared. Mix standard was prepared by mixing the 0.5, 1, 1.5, 2 and 2.5 mL of standard (A) and 1, 2, 3, 4 and 5 mL of standard (B) then made up the volume upto 10 mL with mobile phase. Samples in triplicates were made for each concentration and peak areas were plotted against the corresponding concentrations to obtain the calibration graphs.

### 2.3. Sample preparation

Twenty tablets were weighed and crushed to a fine powder. Tablet powder equivalent to 5 mg of AMLB and 40 mg of TEL was accurately weighed and transferred to a 100 mL volumetric flask. To this was added about 50 mL of methanol and flask was sonicated for 15 min. The flask was shaken, and the volume was made up to the mark with methanol. The above solution was then filtered through 0.45 μ Whatman filter paper and the filtrate was then appropriately diluted with mobile phase to get a final concentration of 5, 15, 30 μg/mL of AMLB and 20, 40, 60 μg/mL of TEL. Prior injecting the solution in chromatographic system, it was filtered through 0.45 μ HPLC syringe filter. Sample analysis was performed for three replicates and eluent was monitored at 254 nm.

### 2.4. Method validation

The method was validated according to the ICH guidelines. The following validation characteristics were addressed: linearity, accuracy, precision, specificity, limits of detection and quantitation

#### 2.4.1. System suitability testing (SST)

System suitability standard solution which contained 20 μg/mL AMLB and 50 μg/mL TEL were prepared by appropriately diluting and mixing the corresponding stock standard solutions. System suitability was determined from six replicate injections of the system suitability standard before sample analysis. According to the monograph, the acceptance criteria for AMLB were less than 2% R.S.D. and a signal-to-noise ratio of at least ten for the corresponding peak area. For TEL, acceptance criteria were less than 2% R.S.D.

#### 2.4.2. Linearity and range

Standard calibration curves were prepared over a concentration range of 5-30 μg/mL for AMLB and 10-60 μg/mL for TEL. The data of peak area versus concentration were treated by linear least square regression analysis. The standard curves were evaluated for intra-day and inter-analyst linearity.

#### 2.4.3. Accuracy

To study the reliability and suitability of the developed method, recovery experiments were carried out. These studies were performed by standard addition method. Known amounts of standard AMD and MET were added to pre-analyzed samples and its recovery was compared with the theoretical concentration.

#### 2.4.4. Precision

According to the ICH recommendations, precision must be considered at two levels, repeatability and intra-day 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. The intra-analyst precision was determined by giving three different concentrations with six replicates by other analyst.

#### 2.4.5. Limits of detection and quantitation

The limit of detection (LOD) of the method refers to that minimum concentration of the active component that can be effectively estimated based on visual evaluation and the limit of quantitation (LOQ) is the lowest amount of analyte in a sample, which can be quantitatively determined with suitable precision and accuracy.

## 3. Results

### 3.1. Method development and optimization

To develop a suitable method for the estimation of AMLB and TEL, different mobile phases were employed to achieve the best separation and resolution. The method development was initiated with using a mobile phase of potassium dihydrogen phosphate buffer and acetonitrile at various ratios (60:40, 40:60, 50:50) (v/v)) and then changed to sodium dihydrogen phosphate buffer and acetonitrile (60:40, 40:60, 50:50) (v/v)) at different pH and finally, the mobile phase consisting of aqueous sodium dihydrogen phosphate buffer: acetonitrile (40:60 v/v pH-6) mixture was found to be appropriate allowing good separation of compound at a flow rate of 0.8 mL/min using a C 18, 250 mm × 4.6 mm column. In order to obtain a satisfactory and full detection for this new method, UV-vis spectra of standard AMLB and TEL solution were obtained. Based on the highest UV absorbance for AMLB and TEL, 254 nm was chosen. Retention times were 4.0 min and 8.2 min for amlodipine and telmisartan, respectively (Figure 1).

**Table 1.**
Linearity parameters for the simultaneous estimation of AMLB and TEL.

| Parameter | AMLB | TEL |
|---|---|---|
| Linearity range (μg/mL) | 5-30 | 10-60 |
| Slope | 34.29 | 81.43 |
| Intercept | 5.649 | 18.61 |
| Correlation coefficient (*r*) | 0.999 | 0.999 |

### 3.2. Method validation

For the construction of calibration curves, six calibration standard solutions were prepared over the concentration range of 5–30 μg/mL for AMLB and 10–60 μg/mL for TEL. The results, summarized in Table 1, showed a good correlation between analytes peak area and concentration with $r > 0.999$ ($n = 6$).

**Table 2**
Method validation results for studied compounds.

| Parameter | | | AMLB | TEL |
|---|---|---|---|---|
| SST | Area | | 547.80 | 1 780.70 |
| | Retention time | | 3.50 | 8.10 |
| | Theoretical plates | 10% plate | 6 162.65 | 8 552.12 |
| | | 50% plate | 9 300.93 | 13 277.15 |
| | | Asymmetry | 0.66 | 0.63 |
| Validation | Precision (%R.S.D.) | Repeatability | 1.84 | 0.59 |
| | | Inter-day | 1.50 | 0.35 |
| | | Inter-analyst | 1.50 | 0.46 |
| | Accuracy (%R.S.D.) | | 1.80 | 0.54 |
| | Accuracy (%recovery) | | 98.29 | 99.55 |
| | LOD | | 50.00 ng/mL | 2.00 μg/mL |
| | LOQ | | 140.00 ng/mL | 4.00 μg/mL |

**Table 3**
Estimation of AMLB and TEL in SARTEL-AM tablets.

| S.No. | Parameter | Limits | |
|---|---|---|---|
| | | AMLB | TEL |
| 1. | % found | 98.240–101.110 | 99.453 –101.220 |
| 2. | SD | 0.048–0.276 | 0.214–0.089 |
| 3. | RSD | 0.607–0.986 | 0.218–0.535 |

#### 3.2.1. Linearity and range

For the construction of calibration curves, six calibration standard solutions were prepared over the concentration range of 5.0–30.0 μg/mL for AMLB and 10.0–30.0 μg/Ml for TEL. The results, summarized in Table 1, showed a good correlation between analytes peak area and concentration with $r > 0.999$ ($n = 6$).

#### 3.2.2. Accuracy and precision

The acuracy was evaluated by the recovery of AMLB and TEL. The summary of the results and average mean of recovery data for each level of both active pharmaceutical ingredients (API) was within accepted range shown in Tables 2. The average results of repeatability, Inter-day and Inter-analyst of AMLB and TEL was within the limit and R.S.D. was (1.84, 0.590), (1.50, 0.35) and (1.50, 0.46), respectively, which indicated a good precision. Theoretical plates and Asymmetry (As) for System suitability was calculated and shown in table 2.

#### 3.2.3. Sensitivity

The limit of detection and limit of quantitation decide about the sensitivity of the method. Tests for the procedure were performed on samples containing very low concentrations of analytes based on the visual evaluation method. In this method, LODis determined by the analysis of samples with known concentration of analyte and by establishing the minimum level at which the analyte can be reliably detected. Accordingly, the LOQ is determined by the analysis of samples with known concentration of analytes and by establishing the minimum level at which the analyte can be quantified with acceptable accuracy and precision (R.S.D. <2%). The LOD and LOQ values were found to be 50 and 140 ng/mL for AMLB and 2.0 and 4.0 μg/mL for TEL.

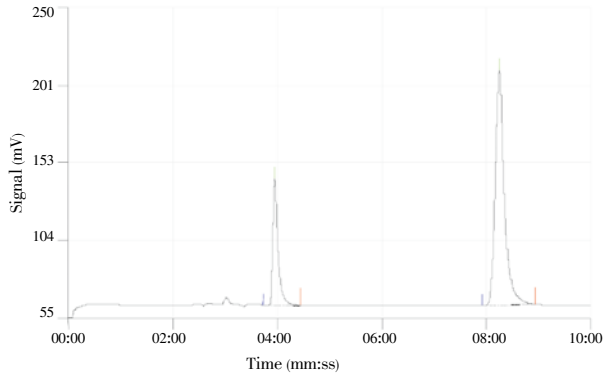


**Figure 1.** Typical chromatogram of AMLB (RT : 4) and TEL (RT : 8.1).

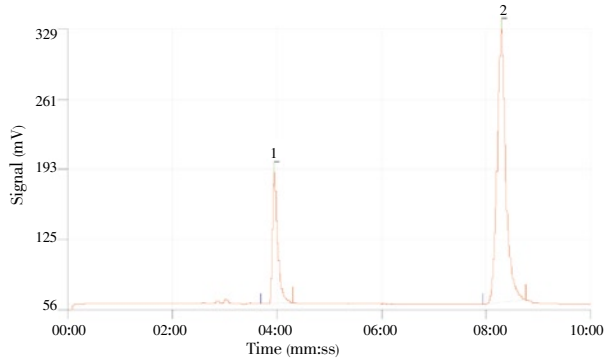


**Figure 2.** Typical chromatogram of AMLB (RT : 4) and TEL (RT : 8.1) in SARTEL-AM tablet.

### 3.3. Label claim recoveries from SARTEL-AM tablets

The proposed method was evaluated in the assay of commercially available tablets containing 40 mg of TEL and 5 mg of AMLB. Six replicate determinations ($n$=6) were carried out on an accurately weighted amount of the pulverized tablets equivalent to 40 mg of TEL and 5 mg of amoldipine as amoldipine besylate. The label claim found was to be 99.45%–101.22% of TEL and 98.24%–101.11% of AMLB per tablet (Table 3) (Figure 2).

## 4. Discussion

Validation of an analytical method is the process by which it is established by laboratory studies, and the performance characteristics of the method meet the requirements for the intended analytical application. Validation is required for any new or amended method to ensure that it is capable of giving reproducible and reliable results, when used by different operators employing the same equipment in the same or different laboratories. The type of validation programme required depends entirely on the particular method and its proposed applications. The precision of an analytical method is the degree of agreement among individual test results obtained when the method is applied to multiple sampling of a homogenous sample. Precision

is a measure of the reproducibility of the whole analytical method (including sampling, sample preparation and analysis) under normal operating circumstances. Accuracy indicates the deviation between the mean value found and the true value. It is determined by applying the method to samples to which known amounts of analyte have been added. These should be analysed against standard and blank solutions to ensure that no interference exists. The accuracy is then calculated from the test results as a percentage of the analyte recovered by the assay. Accuracy and precision are not the same a method. They can have good precision and yet not be accurate. Linearity is the ability of the method ability to obtain results which are either directly, or after mathematical transformation proportional to the concentration of the analyte within a given range. This is determined by calculating the regression line using a mathematical treatment of the results (*i.e.* least mean squares) *v.s.* analyte concentration. The range of the method is the interval between the upper and lower levels of an analyte that have been determined with acceptable precision, accuracy and linearity. It is determined on either a linear or nonlinear response curve. Limit of detection is the lowest concentration in a sample that can be detected, but not necessarily quantitated, under the stated experimental conditions. The limit of detection is important for impurity tests and the assays of dosages containing low drug levels and placebos. Limit of quantitation is the lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy. 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 AMLB and TEL in pharmaceutical dosage forms. Moreover, the lower solvent consumption (0.8 mL/min) along with the short analytical run time of 10.0 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.

## Conflict of interest statement

We declare that we have no conflict of interest.

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