Development and Validation of RP-HPLC Method for Estimation of Amlodipine Besylate and Celecoxib in Pharmaceutical Formulation

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ABSTRACT

A simple, precise, accurate, and rapid reverse phase-high performance liquid chromatography (RP-HPLC) method with UV-Visible detector has been developed and subsequently validated for the simultaneous determination of amlodipine besylate (AML) and celecoxib (CEL) in their combined tablet dosage form. The separation was based on the use of a Flowsil C18 analytical column (250 × 4.6 mm, i.d., 5 µm). The mobile phase consisted of a mixture of 80 volumes of acetonitrile and 20 volumes of water. The chromatography was performed by isocratic elution at a flow rate of 1 mL/min. Analytes were detected at 250 nm with linear calibration curves at concentration ranges of 2-12 µg/ml and 50-300 µg/ml for AML and CEL respectively. The retention time of AML and CEL were 1.98 and 3.15 min respectively. The recoveries obtained were 99.46–101.36% for AML, 99.57–101.42% and 99.96–100.87% for CEL. The method was validated according to International conference of harmonisation guidelines in terms of accuracy, precision, specificity, robustness, limits of detection and quantitation, and other aspects of analytical validation. The developed method was applied successfully for HPLC analysis of commercial pharmaceutical products including AML and CEL.

Keywords: Amlodipine besylate; Celecoxib; RP-HPLC.

INTRODUCTION

Amlodipine Besylate (AML) is the besylate salt of amlodipine chemically, 3-ethyl 5-methyl 2-[2-(aminoethoxy)methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-Clearboxylate-benzene sulfonic acid is a synthetic dihydropyridine with anti-hypertensive and antianginal effects. AML inhibits the influx of extracellular calcium ions into myocardial and peripheral vascular smooth muscle cells, thereby preventing vascular and myocardial contraction. These results in a dilatation of the main coronary and systemic arteries, decreased myocardial contractility, increased blood flow and oxygen delivery to the myocardial tissue, and decreased total peripheral resistance. This agent may also modulate multi-drug resistance (MDR) activity through inhibition of the p-glycoprotein efflux pump. Celecoxib (CEL) 4-[5-(4-Methylphenyl)-3(trifluoromethyl)pyrazol-1-yl]benzenesulfonamide is nonsteroidal anti-inflammatory drug. Celecoxib selectively inhibits cyclo-oxygenase-2 activity (COX-2); COX-2 inhibition may result in apoptosis and a reduction in tumor angiogenesis and metastasis. Both Drugs are official in IP5 and BP6. With hypertension osteoarthritis (OA) is another cause of disability in elderly populations. The prevalence of OA is increasing and many studies reported that hypertension is self-regulating risk factor for the occurrence of knee OA. Elderly and obese patients are generally diagnosed as having both hypertension and OA. Hence, a fixed-dose oral formulation of amlodipine and celecoxib has been approved for the control of hypertension and OA. AML (Fig. 1) is a long-acting dihydropyridine derivative calcium-channel blocker utilized for the management of hypertension and angina. Celecoxib (CEL; Fig. 2) is a selective cyclooxygenase-2 inhibitor used for the management of chronic inflammatory and pain problems, such as rheumatoid arthritis and OA. CEL is preferred over other regular nonsteroidal anti-inflammatory drugs (NSAIDs) for its protective action on the gastrointestinal tract and kidney, as it conserves the physiologically beneficial COX-1 in these organs. In addition, it has less of an effect on hypertension when compared to naproxen, ibuprofen, and other NSAIDs. Many analytical
methods reported for estimation AML either single or in combination with other drugs in API, pharmaceutical formulations and biological fluids includes spectrophotometric\(^7\)\(^-\)\(^9\)\(^-\)\(^12\) and HPLC\(^12\)\(^-\)\(^14\). For CEL few analytical methods reported for its estimation in pharmaceutical formulation either alone or in combination with other drugs which includes spectrophotometric\(^15\)\(^-\)\(^16\) and HPLC\(^16\)\(^-\)\(^18\). Literature survey revealed few UV\(^19\)\(^-\)\(^22\) and HPLC\(^23\)\(^-\)\(^24\) methods methods reported for simultaneous estimation of AML and CEL in their dosage forms. Obviously, HPLC methods are superior in compared to spectrophotometric methods in simultaneous quantification. The reported HPLC methods have drawbacks of long retention times for drugs and complex mobile phase composition. As long retention time need a more consumption of mobile phase. So, there is a need of a HPLC method, where the retention time is less and mobile phase having simple in composition.

The purpose of this study was to develop a simple, rapid, precise, and accurate RP-HPLC method for the simultaneous estimation of these drugs in combined tablet dosage form.

**Preparation of Stock and Standard Solutions**

A sample of 25 mg of each drug is weighed and transferred to a 25-ml volumetric flask; 15 ml of acetonitrile is added, and the solution is sonicated for 15 min. The volume is made up to the mark with acetonitrile to obtain a stock solution of 1000 µg/ml. From the standard stock solutions, 2.5 ml are withdrawn and transferred to 25 ml volumetric flasks, and the volume is made up to the mark with mobile phase to obtain working standard solutions of 100 µg/ml.

**Preparation of Sample Solution**

The assay of commercial tablets was established with present chromatographic condition developed and it was found to be more accurate and reliable. To determine the content of AML and CEL in conventional tablet (5 mg AML/200 mg CEL) twenty tablets were weighed; their mean weight was determined and was finely powdered. Tablet powder equivalent to 10 mg AML with relevant quantities of CEL was weighed and transferred to a 100 ml volumetric flask, extracted for 30 mins with acetonitrile and volume was made up to 100 ml with acetonitrile. 0.5 ml of above solution was taken in 10 ml volumetric flask and volume was made up to 10 ml with mobile phase, and final solution (5 µg AML, 200 µg CEL/ml) was filtered through 0.45 μ millipore filter and it was analyzed by HPLC system.

**Method Validation**

The developed method was validated according to the ICH guideline\(^25\). Method validation included system suitability test, stability, and the validation parameters involving specificity, linearity, accuracy, precision and robustness.

### EXPERIMENTAL

**Materials and Reagents**

Samples of Amlodipine Besylate and Celecoxib were obtained as gift sample from RA Chem Ltd, Hyderabad Pvt. Ltd (India). Tablet formulation CONSSENSI containing Amlodipine Besylate (5 mg) and Celecoxib (200 mg) were procured from commercial market. All the solutions were protected from light and were analyzed on the day of preparations. Glass wares used in each procedure were soaked overnight in a mixture of chromic acid and sulphuric acid rinsed thoroughly with double distilled water and dried in hot air oven. All the reagents were of analytical-reagent (AR) grade unless stated otherwise. Millipore water, HPLC grade methanol, acetonitrile were procured from Merck, India. Borosilicate (Class – A) glass wares were used.

**Instruments**

HPLC analysis was carried out using a reversed-phase column-based high performance liquid chromatography method (CYBERLAB, USA). The system consisted of LC-P100 binary pump, and a variable wavelength programmable LC-UV 100 detector. Rheodyne injector fitted with a 2.0 µL loop was used and data were recorded and analysed using LC solutions software version 4.0. Weighing was done on Digital Microbalance (SHIMADZU AUX 220). Ultra sonicator (Citizen ultra sonicator) was used for sonicating the drug and sample solution.

**Chromatographic Conditions**

The chromatographic separation was carried out on the Flowrosil C18 column (250 x 4.6 mm, 5-µm particle). The mobile phase consisted of acetonitrile: water in the ratio of 80: 20 v/v. The injection volume was 20 µL and eluents detected at 250 nm with the flow rate 1.0 ml/min. The HPLC system was operated at a room temperature of 30°C.

**RESULTS AND DISCUSSION**

Optimization of chromatographic condition

From the UV spectra it was observed that AML and CEL have a considerable absorbance at 250 nm. So, 250 nm was selected as detection wavelength. Various combination of mobile phase consisting of acetonitrile, methanol and water. Preliminary experiments were carried out with different combinations of acetonitrile or methanol with water was to separate the peaks of AML and CEL and to obtain suitable retention times and peak symmetry. Finally, a mobile phase consisting of acetonitrile and water in the ratio of 80:20, v/v and Flowrosil C18 column (250 x 4.6 mm; 5 µm) were selected to achieve good resolution and acceptable peak symmetry. Flow rates between 0.5 and 1.2 ml/min were tried. Flow rate of 1.0 ml/min was observed to be enough to get both the drugs eluted within less than 10 min. The optimized chromatogram Fig. 3. The retention time of AML and CEL were found to be 1.98 and 3.15 min respectively.

![Figure 1: Structure of AML](image1.png)

![Figure 2: Structure of CEL](image2.png)

![Figure 3: Chromatogram of standard solution AML and CEL from binary mixture.](image3.png)
Method Validation

System Suitability Test

After setting the optimum conditions, system suitability parameters for the developed method were determined and compared with recommended limits. System suitability parameters of the method were demonstrated in Table 1. According to results, all of the system suitability parameters were within the recommended limits and the method was found to be suitable for the analysis.

| Parameter                  | Criteria          | AML     | CEL     |
|----------------------------|-------------------|---------|---------|
| Capacity factor ($k'$)    | $k' > 2$          | 2.824   | 4.321   |
| Tailing factor ($T$)      | $T < 2$           | 1.19    | 1.07    |
| Theoretical plates ($N$)  | $N \geq 2000$     | 6380    | 9482    |
| Resolution ($R_s$)        | $R_s > 2$         | -       | 4.02    |
| % RSD (peak area)         | % RSD $\leq 1$   | 0.74    | 0.52    |

Stability of sample solution

The sample solution stability was analyzed by injecting the same solution at 0, 12, 24, and 48 h. Identical change was not observed in the developed method. Also, results were found within acceptable limits (RSD < 2), which are summarized in Table 2.

| Time (hr) | Assay (%)   | % Difference |
|-----------|-------------|--------------|
|           | AML         | CEL          | AML | CEL |
| Initial   | 100.08      | 99.96        | --- | --- |
| After 12 hr| 100.02    | 99.92        | 0.05 | 0.04 |
| After 24 hr| 99.87     | 99.62        | 0.21 | 0.34 |
| After 36 hr| 99.16     | 99.03        | 0.92 | 0.93 |
| After 48 hr| 98.32     | 98.11        | 1.76 | 1.85 |

Linearity

The linearity ranges for AML and CEL were found to be 2-12 µg/ml and 50-300 µg/ml respectively. The linear regression equation for AML was found to be $y = 7486.5x - 55$ with correlation coefficient 0.9993. The linear regression equation for CEL was found to be $y = 3080.6x - 18600$ with correlation coefficient 0.9991. The data for AML and CEL was shown in Table 3. The calibration curve of AML and CEL were shown in Fig. 4 and Fig. 5 respectively. Linear regression of data from the calibration curve indicated a linear response over the concentration range of both drugs. The curve can therefore be used for determination of AML and CEL in pharmaceutical formulation.
Table 3: Spectral and statistical data for determination of Amlodipine Besylate and Celecoxib by proposed RP-HPLC method.

| Analyte | Detection wavelength (nm) | Linearity range (µg/ml) | Coefficient of determination ($r^2$) | Regression equation ($Y$) | Slope (m) | Intercept (c) | Limit of detection, LOD (µg/ml) | Limit of quantitation, LOQ (µg/ml) |
|---------|--------------------------|------------------------|-----------------------------------|--------------------------|-----------|--------------|----------------------------------|-----------------------------------|
| AML     | 250                      | 2-12                   | 0.9993                            | $Y= 7486x - 55$          | 149.73    | -55          | 0.09                             | 0.29                              |
| CEL     | 250                      | 50-300                 | 0.9991                            | $Y= 3080.6x - 18600$    | 3080.6    | -18600       | 0.24                             | 0.75                              |

$Y = mx + c$, where $x$ is the concentration (µg/ml)

Sensitivity

The sensitivity of the analytical method was evaluated by determining the limits of detection (LOD) and quantitation (LOQ). The values of LOD and LOQ for AML and CEL are given in Table 3. The low values of LOD and LOQ indicates the sensitivity of method.

Precision

The % RSD for intra-day and inter-day precision studies were obtained from three different concentrations (2, 4 and 6 µg/ml for AML and 100, 200 and 300 µg/ml for CEL) within linearity range. The % RSD values for intra-day and inter-day precision were below 1.5 %, indicated that the method was sufficiently precise, as shown in Table 4.

Table 4: Precision Studies.

| Drug | Amount(µg/mL) | Intra-day(n=3) | Inter-day(n=3) |
|------|---------------|----------------|----------------|
|      |               | Amount found | %RSD | Amount found | %RSD |
|      |               | Mean±SD |      | Mean±SD |      |
| AML  | 2             | 1.98±0.024 | 1.23 | 2.06±0.028 | 1.40 |
|      | 4             | 4.02±0.035 | 0.89 | 4.09±0.064 | 1.63 |
|      | 6             | 5.98±0.056 | 0.94 | 5.94±0.067 | 1.14 |
| CEL  | 100           | 100.12±0.39 | 0.39 | 99.74±0.96 | 0.97 |
|      | 200           | 200.86±2.26 | 1.13 | 200.79±1.46 | 0.73 |
|      | 300           | 299.77±3.89 | 1.30 | 299.63±2.54 | 0.85 |

Accuracy

Accuracy was performed by recovery studies using standard addition method. Standard drugs in the range of 50, 100 and 150 % of the sample concentration were added into the sample solution. Each concentration was analyzed in triplicate. Results of recovery studies were found to be in between 98 to 102 % for both AML and CEL, as shown in Table 5.

Table 5: Recovery studies (n = 3).

| Recovery level | AML | CEL |
|----------------|-----|-----|
|                | Amount Added (µg/ml) | Amount Recovered (µg/ml) | % Recovery | Amount Added (µg/ml) | Amount Recovered (µg/ml) | % Recovery |
| 50 %           | 2.5 | 2.49 | 99.62 | 100 | 100.22 | 100.22 |
| 100 %          | 5   | 4.99 | 99.80 | 200 | 198.96 | 99.48 |
| 150 %          | 7.5 | 7.47 | 99.66 | 300 | 299.25 | 99.75 |
Robustness

Robustness study was performed by making small variations in method parameters to assess whether the response is influenced by the small changes. The method parameters investigated in this study are change in the flow rate (± 0.2 ml/min), organic composition of mobile phase (± 5 % v/v) and analytical wavelength (± 2nm). The results were evaluated by recovery values of analyte solutions. Robustness study showed that variations in flow rate, composition of mobile phase and analytical wavelength do not have a significant effect on the analyte response. The robustness data was shown in Table 6.

Table 6: Chromatographic parameter setting applied in the robustness investigation.

| Parameter               | Modification | % Recovery | % RSD |
|-------------------------|--------------|------------|-------|
|                         |              | AML        | CEL   | AML | CEL |
| Flow Rate(ml/min)       | 0.9          | 100.20     | 99.64 | 0.67 | 0.88 |
|                         | 1.0          | 99.92      | 99.38 | 1.21 | 0.94 |
|                         | 1.2          | 99.45      | 99.82 | 0.82 | 0.55 |
| Mobile Phase (acetonite:water) | 85 : 15 v/v | 99.74      | 99.34 | 0.84 | 0.71 |
|                         | 80:20 v/v    | 99.49      | 99.66 | 0.91 | 0.86 |
|                         | 75:25 v/v    | 99.68      | 99.49 | 0.72 | 0.75 |
| Wavelength(nm)          | 248          | 99.84      | 99.63 | 0.87 | 1.32 |
|                         | 250          | 99.92      | 99.71 | 1.25 | 1.01 |
|                         | 252          | 99.89      | 99.85 | 1.41 | 0.46 |

Specificity

Specificity is the ability to unequivocally assess the analyte in the presence of components that may be expected to be present. Typically, these might include impurities, degradants or matrix. Specificity of an analytical method is its ability to accurately and specifically measure the analyte of interest without interference from blank or placebo. The peak purities of AML and CEL were assessed by comparing the retention times of standard AML and CEL and the sample, and good correlation was obtained between the retention time of the standard and sample. Placebo and blank were injected and there were no peaks. There is no interference of degradation peaks on drug peaks hence, the method is specific.

Analysis of commercial formulation

The proposed method was applied for the determination of Amlodipine Besylate and Celecoxib in marketed formulations available (CONSENSI TABLETS). The % recovery (Table 7) was found to be 100.08±0.18 and 100.25±0.45 for Amlodipine Besylate and Celecoxib respectively.

Table 7: Analysis of Amlodipine Besylate and Celecoxib in commercial formulation

| Formulation      | Labelled claim(mg) | Amount found*(mg) | %Recovery±%RSD |
|------------------|--------------------|-------------------|----------------|
|                  | AML | CEL | AML | CEL | AML | CEL |
| CONSENSI TABLETS | 5   | 200 | 5.04 | 200.50 | 100.08 ± 0.18 | 100.25 ±0.45 |

*Average of three determinations

CONCLUSION

The proposed method for the estimation of amlodipine besylate and celecoxib was validated as per the ICH guidelines and it is simple, specific and economical. Furthermore, this simple and rapid RP-HPLC method can also be used successfully for the determination of amlodipine besylate and celecoxib in pharmaceutical formulations without any interference from the excipient.

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CONFLICT OF INTEREST: There are no Conflicts of interest

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