Application of Dual Wavelength Spectrophotometric Method for Quantification of Amlodipine Besylate and Celecoxib in their Combined Dosage Form

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ABSTRACT

Objectives: The present study focused on application of a simple, sensitive, rapid, accurate and precise, dual wavelength spectrophotometric method for the quantification of amlodipine besylate and celecoxib in combined dosage form using methanol as a solvent. Materials and Methods: Based on overlain spectra, for quantification of both drugs by proposed dual wavelength spectrophotometric method, the wavelengths selected for the analysis are 280 nm (λ1), 290 nm (λ2), 340 nm (λ3) and 360 nm (λ4). For the quantification of amlodipine besylate absorbance difference measurements was carried out at 340 nm and 360 nm where celecoxib shows same absorbance value. In case of celecoxib absorbance difference measurement carried out at 280 nm and 290 nm where amlodipine besylate showed the same absorbance value at both the wavelengths. The proposed was validated in accordance with guidelines of ICH. Results: The method shown linearity in the concentration range 20-100 µg/ml and 5-25 µg/ml for celecoxib and amlodipine besylate respectively, with good correlation coefficient (r²>0.998). The percentage recovery at different concentration levels were found to be 98.33 to 99.75 % for amlodipine besylate and 98.58 to 99.303 % for celecoxib, indicating the accuracy of the method. Conclusion: The results of analysis were statistically validated and demonstrated to be free from interferences through recovery studies. For the quantification of both the drugs in the commercial available tablets the method was successfully applied. Key words: Absorbance difference, Amlodipine besylate, Celecoxib, ICH, Accuracy.

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-carboxylate-benzene sulfonic acid [Figure 1] is a synthetic dihydropyridine with antihypertensive 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. This 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 [Figure 2] is a 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 IP and BP. 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 a self-regulating risk factor for the occurrence of knee OA. Elderly and obese patients are usually diagnosed with both hypertension and OA. A fixed-dose oral formulation of amlodipine and celecoxib has thus been approved for the control of hypertension and OA. AML is a dihydropyridine derivative calcium-channel blocker used in the management of hypertension and angina. Celecoxib (CEL) is a selective cyclooxygenase-2 inhibitor used for the management of chronic inflammatory and pain problems, such as rheumatoid arthritis and OA. CEL has protective action on the gastrointestinal tract and kidney with physiologically beneficial, hence it is preferred over other regular nonsteroidal anti-inflammatory drugs (NSAIDs). Furthermore, when compared to other NSAIDs it has fewer effects on hypertension. Numerous methods of analysis have been reported for estimation AML either single or in combination with other drugs in the API, pharmaceutical formulations and biological fluids includes spectrophotometric and HPLC.

Figure 1: Structure of AML.
wavelength spectroscopy provides an efficient method for analyzing a component in the presence of an interfering component. For elimination of interferences, dual analytical wavelengths were selected in a way to make the absorbance difference zero for one drug in order to analyze the other drug. Literature review suggests, there are no reports available based on dual wavelength UV spectrophotometric method for the estimation of AML and CEL in their combined dosage form. The authors therefore made an attempt to develop a simple, economical, accurate, precise and sensitive dual wavelength UV spectrophotometric method for simultaneous determination of AML and CEL in their combined dosage forms. The proposed methodology has been validated in accordance with the ICH guidelines and its updated international convention.

MATERIALS AND METHODS

Instrumentation and chemicals
A double beam UV-visible spectrophotometer (LABINDIA) having two matched quartz cells with 1 cm light path and loaded with UV WINS software version 5.2.0.1104. was used for recording of spectra and measuring absorbance. An electronic analytical weighing balance (SHIMADZU AUX 220) was used for all weighings and a Ultrasonicator (Citizen,India) used for the sonication of all analytical solutions. Pure samples of AML, CEL were obtained as gifted samples from pharma industry. Tablet formulation CONSENSI containing Amlodipine Besylate (10 mg) and Celecoxib (200 mg) were procured from commercial market and a Whatman filter paper no. 41 (Whatman International Ltd., England) was used. All the chemicals used were of analytical grade purchased from SD Fine Chemicals, Mumbai. Double distilled water was used for all studies.

Preparation of Standard stock Solutions (100 μg/ml)
The preparation of standard stock solution (1 mg/ml) each of AML and CEL were done using methanol as solvent and further dilutions were carried out with methanol for both stock solutions to get a concentration of 100 μg/ml each. These solutions were used as working standard solutions for further analysis.

Preparation of Sample Solution
Twenty tablets each claimed to contain 10 mg AML and 200 mg CEL were triturated to a fine powder after weighing accurately. From the powder, a quantity equivalent to 10 mg AML and 200 mg CEL was accurately weighed and transferred to 100 ml volumetric flask containing methanol and sonicated for 15 min. The volume was adjusted to the level with methanol and filtered through whatman filter paper no 41. Transferred, 0.4 ml from the above was prepared solution to a 10 ml volumetric flask and volume was adjusted with methanol to give a solution containing 4 μg/ml AML and 80 μg/ml CEL. With this solution analysis was carried for the estimation of AML and CEL. The responses of the sample solution were measured at 280 nm (λ₁), 290 nm (λ₂), 340 nm (λ₃) and 360 nm (λ₄) for quantification of CEL and AML respectively. The amount of AML and CEL present in the sample solution were determined by substituting the absorbance into the regression equation for AML and CEL respectively.

RESULTS AND DISCUSSION

Selection of the wavelengths
From the appropriate dilution of working standard stock solution 100 μg/ml AML and 100 μg/ml of CEL were separately prepared and scanned in UV range of 200-400 nm. The zero order spectra were obtained as shown in Figure 3. From the overlain spectra, four wavelengths 280 nm (λ₁), 290 nm (λ₂), 340 nm (λ₃) and 360 nm (λ₄) were selected for quantitation of both the drugs by proposed dual wavelength spectrophotometric method. The quantitative determination of AML was carried out by measuring the absorbance difference at 340 nm and 360 nm where CEL shows same absorbance value. The quantitative determination of CEL was carried out by measuring the absorbance difference at 280 nm and 290 nm where AML showed same absorbance value at both the wavelengths.

Linearity and Range
Linearity studies of the method was carried out by diluting appropriate aliquots of working standard solution of AML and CEL into a series of 10 ml volumetric flask with methanol to get a concentration range of 5-25 μg/ml for AML and 20-100 μg/ml for CEL. The absorbance difference of each of the solution measured at selected wavelengths and plotted against concentration to obtain calibration curve. The statistical parameters of the calibration curve for both drugs were shown in Table 1.

Precision
The precision was measured in terms of method precision and intermediate precision. The precision of the method was verified by measuring the absorbance difference of solutions of AML (15 μg/ml) at
340 nm, 360 nm and CEL (60 µg/ml) at 280 nm, 290 nm repeatedly, by the proposed method without changing any parameter. The results were expressed as % RSD. For the proposed method, the intra-day and inter-day precision studies were carried out by analyzing the corresponding responses 3 times on the same day and on 3 different days for different concentrations of standard solutions of AML (10, 15, 20, µg/ml) and CEL (40, 60, 80 µg/ml). The small value of percent RSD (< 2 %) suggests that proposed method is repeatable. The results of intra-day and inter-day precision of AML and CEL are Table 2.

### Accuracy

The accuracy of the method was determined by computing recovery of AML and CEL using standard addition method. Known amounts of standard solutions of AML (2, 4 and 6 µg/ml) and CEL (64, 80, 96 µg/ml) were added to pre-quantified sample solutions of AML (4 µg/ml) and CEL (80 µg/ml). By applying the regression equation of the calibration curve the amount of AML and CEL recovered was determined. The accuracy was repeated for three times at each level. The recovery study for each level was carried out for three times and % mean recovery was calculated and results are shown in Table 3. The results of percentage recovery studies were the accuracy of the the proposed method.

### Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD and the LOQ of the AML and CEL were calculated using the following equations as per ICH guidelines.

\[
LOD = 3.3 \times \sigma/S \\
LOQ = 10 \times \sigma/S
\]

Where,  
\( \sigma \) = The Standard deviation of y-intercepts of calibration curves.  
\( S \) = Average slope of calibration curve.

LOD values for AML and CEL were found to be 1.054 and 0.101 µg/ml, respectively and LOQ values for AML and CEL were found to be 3.196 and 0.308 µg/ml, respectively. These data reveal that proposed method is sensitive for the determination of AML and CEL.

### Assay of the pharmaceutical formulation:

The developed, validated dual wavelength spectrophotometric method was successfully applied for the quantification of AML and CEL in their combined dosage form. The method is found to be appropriate for simultaneous estimation of AML and CEL as indicating from the assay results obtained were in accordance with the corresponding labelled amounts and free from interference from excipients normally present in tablets. The results are given in Table 4.

### CONCLUSION

The proposed dual wavelength method produces accurate and precise results for determining AML and CEL in marketed formulation (tablet) without prior separation and is readily applicable for routine analysis. The most striking feature of the dual wavelength method is its simplicity and rapidity. Method validation has been demonstrated by a variety of tests for linearity, accuracy and precision. The proposed approach has been successfully applied for the quantification of these drugs in commercial tablets.

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### CONFLICT OF INTEREST

The authors declare no conflicts of interest.
**REFERENCES**


**ABBREVIATIONS**

AML: Amlodipine Besylate; CEL: Celecoxib; RSD: Relative standard deviation.

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**PICTORIAL ABSTRACT**

A simple and rapid dual wavelength based UV spectrophotometric method developed and validated for estimation of amlodipine besylate and celecoxib in combined dosage form.

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**SUMMARY**

The quantitative determination of amlodipine besylate was carried out by measuring the absorbance difference at 340 nm and 360 nm where celecoxib shows same absorbance value. The quantitative determination of celecoxib was carried out by measuring the absorbance difference at 280 nm and 290 nm where amlodipine besylate showed same absorbance value at both the wavelengths. These methods obey Beer’s law in the concentration range 20-100 µg/ml and 5-25 µg/ml for celecoxib and amlodipine besylate respectively with good correlation coefficient (r2>0.998). The results of analysis were validated statistically and by recovery studies and found to be free from interferences.
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