Research Article

Development of UV Spectrophotometric Procedures for Determination of Amlodipine and Celecoxib in Formulation: Use of Scaling Factor to Improve the Sensitivity

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FDA has recently approved a new fixed-dose combination of amlodipine besylate (AMD) and celecoxib (COX) for the treatment of hypertension and osteoarthritis. No analytical method has been reported for analysis of these two analytes so far. Hence, to monitor the quality and quantity in the formulation of AMD and COX a simple, accurate, precise, economical, and eco-friendly spectroscopic analytical method has been established. The first method involves the determination of AMD and COX by the first derivative UV spectroscopic method with scaling factor 10. However, the second method was based on the direct measurement of UV absorbance of AMD at 364.3 nm and ratio first derivative UV spectroscopic method for COX. Both methods showed good linearity in the range of 5 to 40 μg/ml for COX, whereas AMD showed linearity in the range of 0.5 to 10 μg/ml in first derivative method with scaling factor 10 and 1 to 10 μg/ml in the second method with good correlation coefficient ($R^2 > 0.998$). Both the methods were validated for LOD, LOQ, accuracy, precision, recovery studies, and stability as per the ICH guidelines, and the validated results were well within the acceptable range. Both the methods were successfully utilized for the determination of AMD and COX in the presence of each other in the formulation, and statistically compared between the proposed methods. Therefore, the proposed procedures can be utilized for regular quality control studies.

1. Introduction

Hypertension and osteoarthritis (OA) are major health problems in the middle- and old-age population. Generally, these two diseases coexist, and 40% of OA patients were diagnosed for hypertension [1]. However, the treatment for these two diseases is challenging, because of the adverse effects of NSAIDs on the blood pressure [2]. Nevertheless, celecoxib (COX, Figure 1(a)) was found to be a better choice because it has low risk on blood pressure than ibuprofen, naproxen, or other NSAIDs, and COX has low gastrointestinal and kidney toxicity [3, 4]. Hence, FDA has recently approved a fixed-dose combination of calcium channel blocker, amlodipine besylate, and selective COX-2 inhibitor, celecoxib, for the treatment of hypertension and OA [5].

Amlodipine besylate (AMD, Figure 1(b)), a long-acting calcium channel blocker, is a dihydropyridine derivative extensively used for the management of hypertension and angina pectoris [6]. Several analytical methods were described in the literature for quantification of AMD alone and
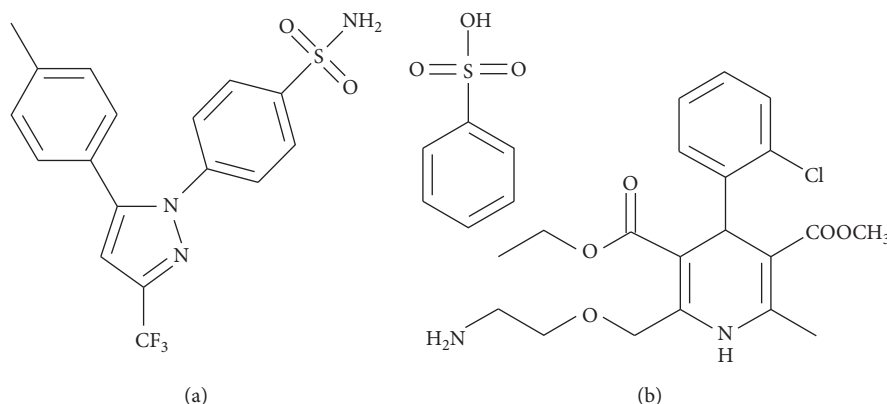
Figure 1: Chemical structure of celecoxib (a) and amlodipine besylate (b).

in combination with other drugs from formulation and biological fluids. These analytical methods include spectrophotometric methods [7–9], spectrofluorometric methods [10], HPTLC [11], RP-HPLC [12–14], LCMS/MS [15], capillary electrophoresis, and electrochemical methods [16–23]. Celecoxib, (COX) selective cox-2 inhibitor, is a potent analgesic, antipyretic, and anti-inflammatory drug. COX is far better than other traditional NSAIDs in terms of gastrointestinal safety profile, by sustaining the physiological amount of prostaglandins in the stomach and kidney. Hence, it is generally preferred in chronic inflammatory diseases such as OA [24, 25] and also been used in chemodefensive activity in different cancers [26]. The medicinal application of COX has been increasing continuously; hence, different analytical methods have also been reported, such as spectrophotometry [27, 28], spectrofluorometry [28, 29], RPHPLC [30–32], LCMS/MS, [33, 34], and capillary electrophoresis methods [35]. However, no analytical technique has been reported for simultaneous assessment of AMD and COX in recently approved pharmaceutical formulation.

UV spectroscopic methods are simple, economical, and accurate. However, for multicomponent formulations consisting of analytes showing a complete overlap of UV absorption spectra, it becomes difficult to estimate the analytes in the presence of each another using zero-order UV spectra without prior separation. However, derivatization of zero-order spectra will allow us to analyze these components in the presence of each other, by measuring the peak amplitude of derivative spectra at zero crossings for one of the analyte. Furthermore, derivatization of ratio spectra can also be used to avoid the interference by another component and tablet excipients [36–40]. Hence, an attempt has been made in this proposal to develop two economical, simple, accurate, and precise derivative UV spectrophotometric methods for simultaneous determination of AMD and COX in pharmaceutical formulations.

2. Experimental

2.1. Chemicals and Reagents.
Pure samples of amlodipine besylate (99.1%) and celecoxib (98.9%) were procured from the Sigma Aldrich (Germany). Analytical-grade ethanol was

purchased from Sigma Aldrich. The distilled water prepared in our laboratory by Milli Q (Millipore, USA) was used throughout the experiments. Amlodipine tablet (10 mg/tablet) and celecoxib capsule (200 mg/capsule) were purchased from the local market. Tablets consisting of AMD : COX in a concentration of 2.5 : 200, 5 : 200, and 10 : 200 mg/tablet, respectively, were prepared in laboratory using 8 mg of lactose, 5 mg of magnesium stearate, 10 mg microcrystalline cellulose, 12.5 mg talc, and 10 mg sodium starch glycolate per tablet.

2.2. Instruments.
Shimadzu UV-Vis spectrophotometer (1700) has been used to record the UV spectra of AMD and COX using 10 mm quartz cuvettes. Scanning of the samples was performed at a speed of 50 nm/min by adjusting the slit width to 1 nm. At high concentration levels of analytes, spectra showed high noise; hence, spectra were smoothened with 8 nm. Calibrated volumetric flasks were used for preparations of stock and working standards and sample solutions.

2.3. Preparation of Stock and Working Solutions.
Stock solutions of AMD and COX standards were prepared by transferring precisely balanced 100 mg of AMD and COX to two separate 100 ml measuring flasks consisting of 50 ml of ethanol. AMD was freely soluble in ethanol, whereas to dissolve COX, the measuring flask was mixed thoroughly for 5 min with the help of sonicator, and then the final volume was adjusted to the 100 ml with ethanol. The working standards were prepared by diluting these solutions with ethanol: water (50% V/V) as a solvent.

2.4. Preparation of AMD and COX Sample Solutions Using Formulation and Laboratory-Prepared Tablets.
Twenty tablets of AMD (10 mg/tablet) were weighed, powdered, and the average weight was calculated. Twenty capsules of COX (200 mg/Capsule) were opened and contents were weighed separately, the average weight was calculated, and then mixed. Both AMD and COX powder equivalent to 10 mg of AMD and 200 mg of COX were transferred to 100 ml

measuring flask. Around 75 ml of ethanol was transferred and sonicated for 15 min at room temperature to dissolve the analytes into the solvent. The solution was filtered and the filter paper was washed with fresh ethanol and the final volume was adjusted with ethanol. Furthermore, the solution was diluted with 50% ethanol to bring the concentration in the range of calibration curve. Similarly, laboratory-prepared tablets were powdered and sample solutions were prepared using the above-mentioned procedure and analyzed.

3. Procedure

3.1. First Derivative Spectrophotometric Method (First Method). Sufficient amount of AMD and COX standard stock solutions were transferred into two separate series of 10 ml volumetric flasks, to get concentration in the range of 0.5–10 μg/ml of AMD (0.5, 2, 4, 6, 8, and 10 μg/ml), and 5 to 40 μg/ml of COX (5, 10, 15, 20, 30 and 40 μg/ml). All these solutions were subjected to the absorption measurement in the range of 200 nm to 400 nm to record the UV absorption spectra using 50% v/v ethanol/water solution as blank. The first derivative spectra were computed for all these spectra using 4 nm as $\Delta\lambda$ with scaling factor 10. The peak amplitude of AMD spectra at zero crossings for COX and peak amplitude for COX spectra at zero crossings for AMD were recorded. The calibration curves were constructed between these amplitude values against the corresponding concentration of AMD and COX separately and respective regression equations were constructed.

3.2. Ratio First Derivative Spectroscopy (Second Method). Sufficient amount of AMD stock solutions was transferred to a sequence of 10 ml measuring flasks containing 20 μg/ml of COX to get the concentration in the level of 1 to 10 μg/ml AMD (1, 2, 4, 6, 8, 10 μg/ml). Similarly, COX standard stock solution was added to a sequence of 10 ml measuring flasks consisting of 2 μg/ml of AMD to get the concentration in the range of 5 to 40 μg/ml of COX (5, 10, 15, 20, 30, 40 μg/ml). UV absorbance spectra were recorded for combined AMD and COX solutions and stored. The absorbance of AMD was measured at 363.4 nm and a calibration curve was constructed for AMD by plotting a graph between absorbance versus concentration. Separately, 2 μg/ml solution of AMD was prepared and the UV spectrum was recorded. The second series of absorbance spectra of AMD and COX were converted to ratio spectra of COX by dividing the combined spectra with UV spectrum of AMD (2 μg/ml). Resulting ratio spectra were converted into the first derivative of ratio spectra of COX by derivatizing with 4 nm as $\Delta\lambda$. The peak amplitude at 286.7 nm was measured, and the graph was constructed by plotting a graph between amplitude value and corresponding concentration. Regression equations were also calculated from calibration curves.

3.3. Application of UV Spectroscopic Methods to the Formulation. Aliquot of sample solution was diluted with 50% ethanol solution to get the working sample solution containing both AMD and COX 1 : 20 μg/ml, 1 : 40 μg/ml, 0.5 : 40 μg/ml, respectively. UV absorption spectra were recorded in the range of 200 nm–400 nm. For the first method, the normal UV spectra of sample solutions were converted into the first derivative using 4 nm as $\Delta\lambda$ with a scaling factor of 10. The concentrations of AMD and COX were determined by recording the peak amplitude at zero-crossing wavelength and corresponding regression equations. In the second method, for determination of AMD, absorbance was measured at 364.3 nm and concentration was calculated using regression equitation. For determination of the concentration of COX, the normal UV spectrum of the formulation was divided by UV spectrum of AMD (2 μg/ml) and the resulted ratio spectrum was converted into a first derivative spectrum using 4 nm as $\Delta\lambda$. The peak amplitude was measured at 286.7 nm, and the concentration of COX was calculated using the corresponding regression equation. Furthermore, both the methods were compared using Student's "t" test and "F" test to know the difference between the analysis results of both methods.

4. Results and Discussion

The UV absorption spectra for AMD and COX in ethanol/water (50% v/v) solution are shown in Figure 2. The UV spectra of COX were completely overlapped by UV spectra of AMD, making it difficult to determine COX in presence of AMD without prior separation by direct UV spectrophotometry. Hence, two derivative UV spectrophotometric methods were developed for simultaneous determination of AMD and COX in formulations. The derivatization of UV spectra could allow us to determine the analytes in the presence of excipients and also remove the interference from the overlapping analyte.

Both the analytes were soluble in ethanol and in the concentration of calibration curve range, working standard solutions were clear solution in the 50% ethanol solution. Hence, 50% ethanol solution was selected as a solvent to develop simple, economical, and eco-friendly UV-spectroscopic methods.

The first method was based on the conversion of normal spectra into the first derivative spectra by differentiating the absorbance spectra of a sample by wavelength by wavelength. Derivative spectra allow us to separate overlapped spectra and also eliminate the interference by excipients and other analytes. This increases the resolution of multicomponent spectra and enhances specificity and sensitivity. Furthermore, this feature allows for the determination of the concentration of one component in the presence of others by measuring the amplitude of derivative spectra at zero-crossing wavelengths, where one of the components will have zero absorbance [36, 37].

The fixed-dose formulations of AMD and COX are available in three different ratios: 2.5 : 200, 5 : 200, and 10 : 200 mg/tablet, respectively. Due to low concentration of AMD in the formulation compared to COX, at zero-crossing wavelength for COX, AMD showed negligible absorbance up to 2 μg/ml due to low amplitude of first derivative spectra. Hence, it was difficult to determine simultaneously both
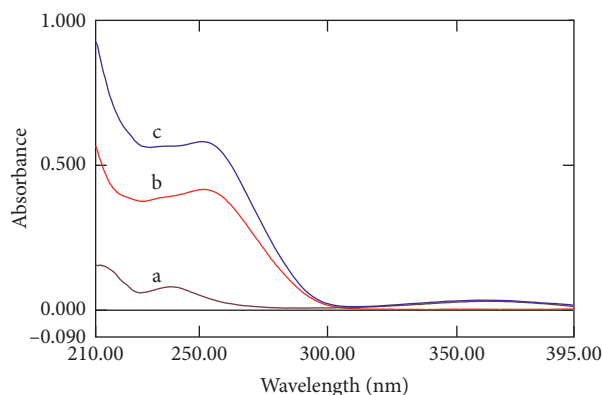
FIGURE 2: Normal UV absorption spectra of AMD (a), COX (b), and mixture (c).

analytes in a formulation containing 2.5 : 200 mg and 5 : 200 mg of AMD and COX. Therefore, with an increase in the concentration, AMD proportionally increases the concentration of COX. Absorption of COX solution, with a concentration above 40 μg/ml, was more than 2.5 and showed noise. Hence, scaling factor of 10 was used during the conversation of spectra to first derivative spectra, which increases the amplitude of the first derivative spectra and allowed to determine the low concentration of AMD from 0.5 μg/ml (Figure 3).

Absorbance spectra were recorded for AMD and COX solutions and converted into first derivative spectra with a scaling factor 10 using 4 nm as $\Delta\lambda$. Different wavelengths 2, 4, 8, and 10 nm were applied as $\Delta\lambda$. However, 4 nm was found to be optimal with scaling factor 10; hence, 4 nm was used as $\Delta\lambda$ throughout the experiment. The first derivative spectra of AMD showed 2 maxima at 233.16 nm and 338.34 nm and 3 minima at −219.68 nm, −249.22 nm, and −393.55 nm (Figure 4); however, COX showed zero crossings at −252.0 nm at which AMD had some absorption amplitude (Figure 5). The first derivative spectra of COX showed 1 maximum at 245.59 nm and 2 minima at −209.8 and −272.08 nm (Figure 6). However, AMD showed zero crossings at −210.56 nm and −289.4 nm where COX had some absorbance (Figure 5). However, the amplitude was good at −210.5 nm but linearity was not good, whereas at −289.4 nm, amplitude is low but showed good linearity with excellent regression coefficient. Hence, wavelengths of −252.0 nm and −289.4 nm were selected for quantification of AMD and COX, respectively. Furthermore, analytes showed the same absorbance in pure and in presence of each other at zero-crossing wavelength (Figure 5).

The second method involves the direct measurement of AMD and ratio derivative method for COX measurement. Since UV spectra of COX showed no absorbance in the range of 320 nm to 400 nm, where AMD had some absorbance, a calibration curve was constructed for AMD by measuring the absorbance at 363.4 nm (Figure 7). However, the spectra of COX was completely overlapped by AMD. Hence, ratio derivative spectroscopic method [38–40] has been adopted for quantification of COX, which is based on the following principle. For two compounds (P and Q) with
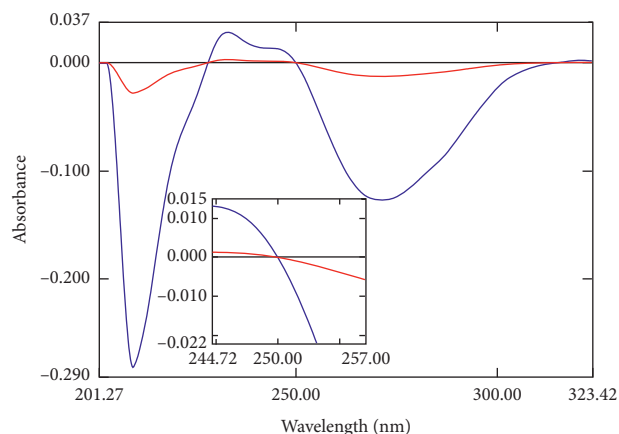

FIGURE 3: First derivative spectra of AMD (0.5 μg/ml) and COX (10 μg/ml) with scaling factor 1 (red) and scaling factor 10 (blue). Enlarged part shows the absorbance difference at 252 nm.
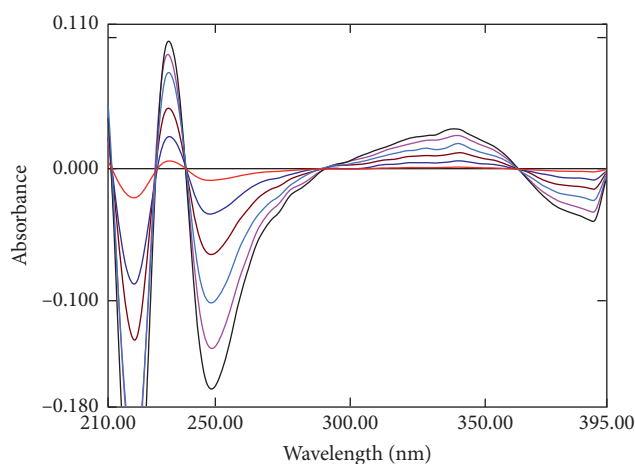

FIGURE 4: The first-order derivative spectra of AMD 0.5 to 10 μg/ml.

no chemical interaction between the compounds and which obey Beers' law for each compound, the following equation (1) can be constructed:

$$A_M = \varepsilon_P C_P + \varepsilon_Q C_Q, \tag{1}$$

where A_M is the absorbance of P and Q mixture, ε_P and ε_Q are molar absorptivity of compounds P and Q, respectively, and C_P and C_Q are the concentration of compounds P and Q, respectively. Dividing the above equation with absorbance equation of one of the pure compound solution spectra ($A_{Q°} = \varepsilon_{Q°} C_{Q°}$) results into the following equation (2):

$$\frac{A_M}{A_{Q°}} = \frac{A_P}{A_{Q°}} + \frac{C_Q}{C_{Q°}}. \tag{2}$$

The expression $C_Q/C_{Q°}$ is constant, which can be eliminated by converting the ratio spectra to derivative spectra. This derivatization generates several maxima and minima, and measurement of peak amplitude at these maxima and minima wavelengths allows us to determine the concentration of one component in the presence of
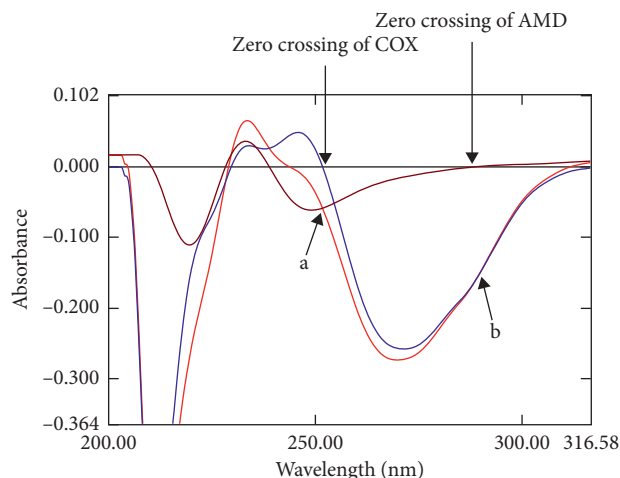
FIGURE 5: The first-order derivative spectra of AMD (brown), COX (blue), and a mixture of AMD and COX (red). (a) AMD ($4\,\mu g/ml$) showing the same absorbance in pure and mixture at zero crossings for COX. (b) COX ($20\,\mu g/ml$) showing the same absorbance in pure and mixture at zero crossings of AMD.
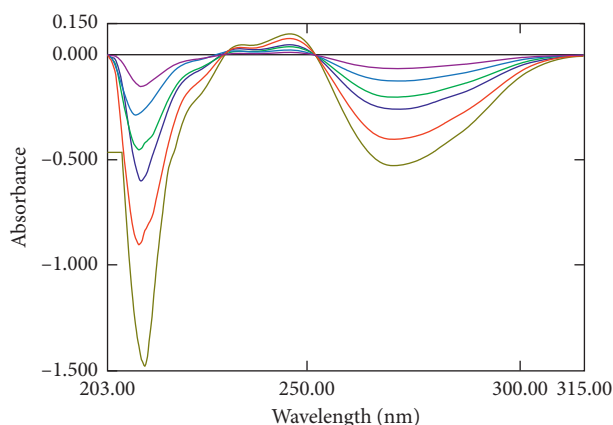

FIGURE 6: The first-order derivative spectra of COX 5 to $40\,\mu g/ml$.

interfering another compound and excipients [38–40]. In this method, UV absorbance spectra of a mixture of AMD and COX were divided by the UV absorbance spectra of AMD solution to generate ratio spectra of COX in the concentration range of 5 to $40\,\mu g/ml$ (Figure 8). Different concentration solutions of AMD were studied (0.5, 2, 4, 8, and $10\,\mu g/ml$) as a devisor; however, devisor concentration of $2\,\mu g/ml$ was selected due to good sensitivity. Ratio spectra were converted into first derivative spectra using 4 nm as $\Delta\lambda$. First derivative spectra (Figure 9) showed two maxima at 222.92 nm and 258.24 nm and three minima at -209.10 nm, -232.35, and -286.7 nm. The peak amplitude measured at these wavelengths was directly proportional to the concentration of COX. However, the amplitude at 222.92 nm, -209.10 nm, and 232.35 nm was low, whereas at 286.7 nm showed good recovery of COX in the laboratory mixed samples with low RSD and good linearity with excellent regression coefficient ($r^2 > 0.999$) when compared to amplitude measured at 258.24 nm. Hence, 286.7 nm was
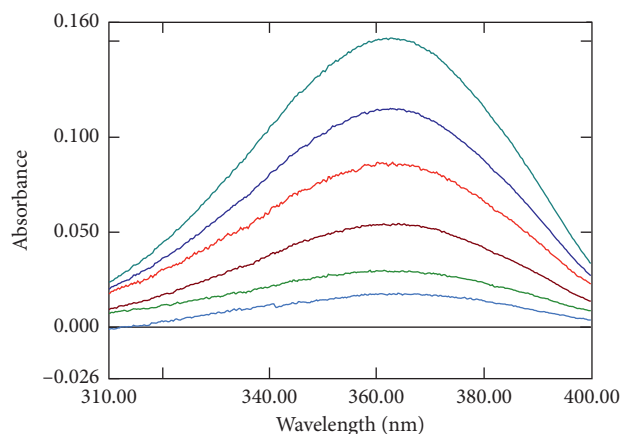
FIGURE 7: Normal spectra of AMD 1 to $10\,\mu g/ml$ (second method).
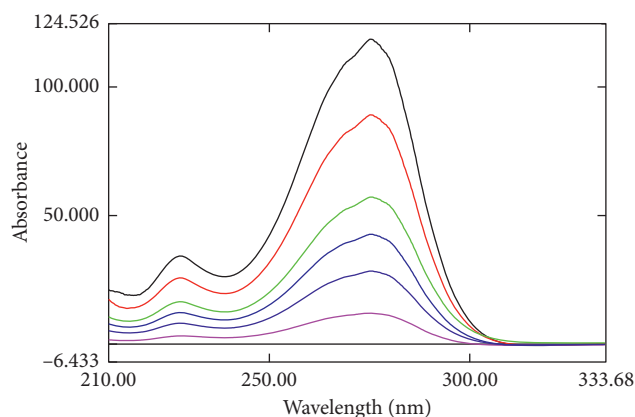
FIGURE 8: Ratio spectra of COX 5 to $40\,\mu g/ml$ using AMD $2\,\mu g/ml$ solution spectrum as a divisor.
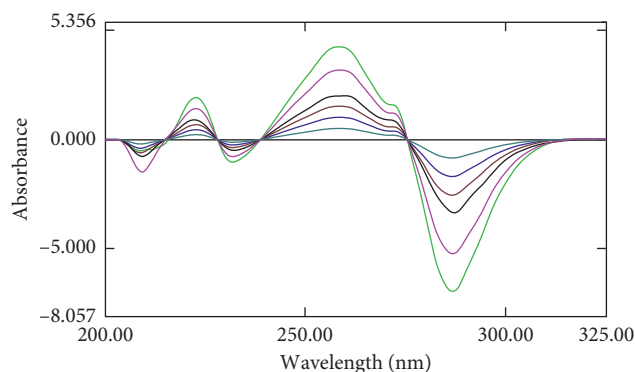
FIGURE 9: First-order ratio derivative spectra of COX 5 to $40\,\mu g/ml$ using 4 nm as $\Delta\lambda$.

selected for analysis of COX. The peak amplitude was measured at 286.7 nm at different concentrations, and a calibration curve was constructed by plotting a graph between amplitude versus concentration of COX. Alternatively, the linearity equation was constructed from the calibration curve.

FIGURE 10: Calibration curves of AML 0.5 to 10 μg/ml, first derivative method (a), 1 to 10 μg/ml in second method (normal absorption at 363.4 nm) (c), COX 5 to 40 μg/ml, first derivative method (b), and second method (first derivative of ratio spectra) (d).

4.1. Method Validation.

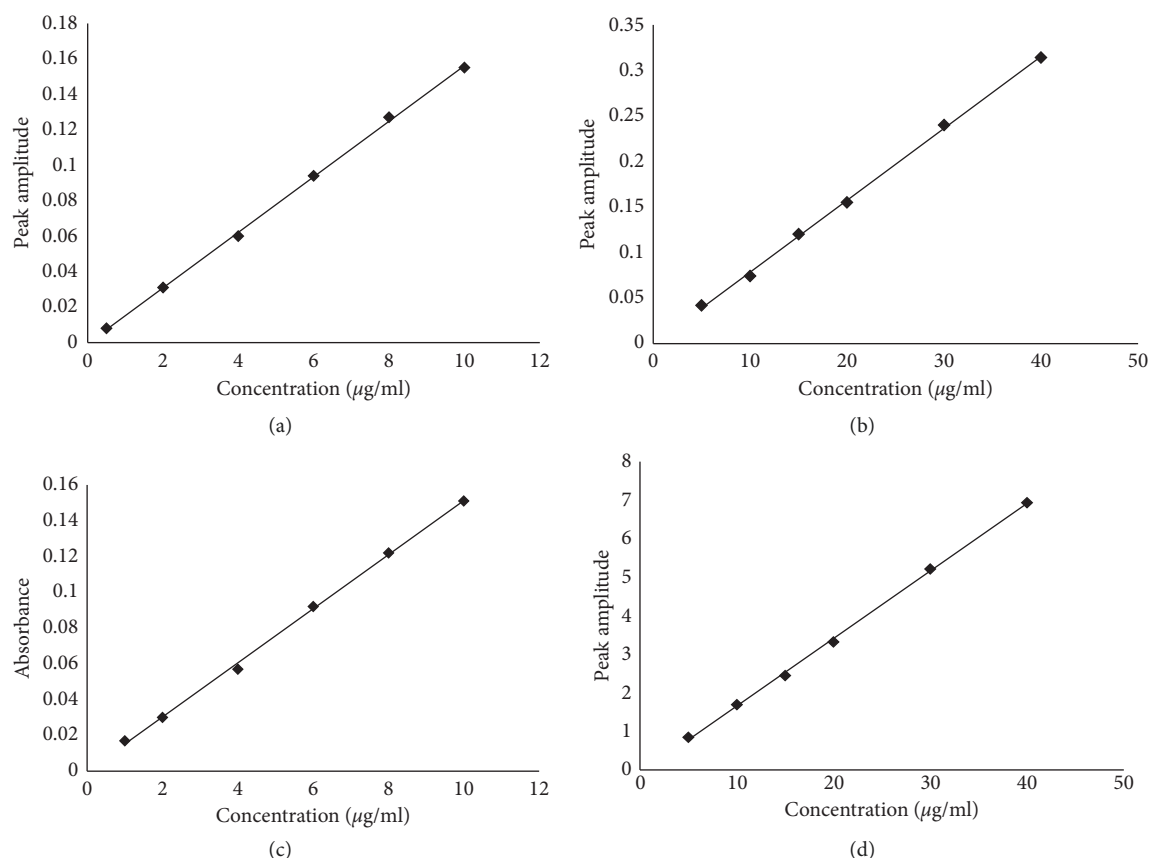Proposed spectroscopic procedures were validated adhering to the ICH guiding principles for linearity, the limit of detection and quantification, accuracy, precision, recovery, selectivity, and stability.

4.2. Linearity.

The linearity range was determined for both analytes by both methods by analyzing the different concentration solutions. AMD exhibited excellent linearity in the concentration of 0.5 to 10 μg/ml in the first method and 1 to 10 μg/ml in the second method with good correlation coefficient ($r^2 > 0.998$). COX was linear in the range of 5 to 40 μg/ml with an excellent correlation coefficient ($r^2 > 0.999$) in both methods. The calibration curves for both analytes by both methods are shown in Figure 10. The linearity range, regression equations, and correlation coefficients are tabulated in Table 1.

4.3. Limits of Detection and Limits of Quantification.

The sensitivity of the spectroscopic methods was established by determining the limits of detection (LOD) and limits of quantification (LOQ). LOD and LOQ were determined according to the ICH guidelines. LOD was determined by 3.3 p/m, whereas LOQ was determined by 10 p/m, where p represents the standard deviation of calibration curve and m represents the slope of the curve. The low LOD and LOQ values showed the good sensitivity of the methods (Table 1).

TABLE 1: Regression equations and validation parameters results for AMD and COX.

Parameters	First derivative method		Second method	
Drugs	AMD	COX	AMD	COX
Wave length (nm)	−252.0	−289.4	363.4	−286.7
Linearity range (μg/ml)	0.5–10	5–40	1–10	5–40
LOD (μg/ml)	0.14	0.45	0.31	0.34
LOQ (μg/ml)	0.42	1.24	0.92	0.99
Slop (m)	0.0157	0.0079	0.0151	0.1753
Intercept (c)	0.0004	−0.0004	0.0006	−0.0947
Correlation coefficient (r^2)	0.9992	0.9990	0.9985	0.9991

4.4. Precision and Accuracy.

The precision of the methods was assessed by interday and intraday precision (Supplementary material File (available here)). For intraday precision, the three different concentrations of both analytes were analyzed by an optimized method in triplicate on the same day. For interday precision, the same solutions were analyzed for three successive days. The precision was expressed as % RSD, whereas accuracy was expressed as % relative error and tabulated in Table 2. The low % RSD (<2.0) and low % RE indicate good precision and accuracy of both methods.

4.5. Recovery Studies.

Recovery studies of the spectroscopic methods were evaluated by the standard addition method

TABLE 2: Precision and accuracy data.

| Amount of drug (μg/mL) | Interday | | | Intraday | | |
	Amount found mean ($n=3$) ± SD	% RSD	% RE	Amount found mean ($n=9$) ± SD	% RSD	% RE
First derivative method						
AMD						
1	1.01 ± 0.01	0.51	0.99	0.99 ± 0.01	1.02	−1.01
4	3.93 ± 0.05	0.41	−1.78	4.01 ± 0.04	1.00	0.25
8	7.94 ± 0.18	1.88	−0.76	7.9 ± 0.15	1.90	−1.27
COX						
5	4.96 ± 0.05	1.01	−0.81	5.08 ± 0.06	1.18	1.57
20	20.16 ± 0.38	1.51	0.79	19.76 ± 0.31	1.58	−1.25
40	39.4 ± 0.66	1.7	−1.52	40.08 ± 0.59	1.47	0.20
Second method						
AMD						
1	0.99 ± 0.01	1.02	−1.01	0.98 ± 0.01	1.02	−2.00
4	4.06 ± 0.07	1.14	1.48	3.94 ± 0.06	2.04	−2.04
8	7.92 ± 0.14	1.97	−1.01	7.86 ± 0.17	2.16	−1.78
COX						
5	4.91 ± 0.03	1.01	−1.83	5.04 ± 0.07	1.39	0.79
20	19.67 ± 0.21	1.39	−1.68	19.81 ± 0.33	1.67	−0.96
40	39.85 ± 0.71	1.33	−0.38	39.4 ± 0.69	1.75	−1.52

SD: standard deviation; % RSD: percent relative standard deviation; % RE: percent relative error.

TABLE 3: Recovery study results by the standard addition method.

| | First derivative method | | Second method | |
	Amount added (μg/ml)	% Recovery	Amount added (μg/ml)	% Recovery
AMD	1	98.87	1	98.49
	2	100.94	2	101.12
	3	99.09	3	99.44
	Across mean	99.63		99.68
	% RSD	1.14		1.33
COX	5	101.23	5	99.17
	10	98.77	10	101.39
	15	99.06	15	99.28
	Across mean	99.69		99.95
	% RSD	1.34		1.25

(Supplementary material File (available here)). Three different concentration solutions of AMD (1, 2, and 3 μg/ml) and COX (5, 10, and 15 μg/ml) were added separately to the previously analyzed formulation solution consisting of 1 μg/ml of AMD and 20 μg/ml of COX. Then, the total amount of AMD and COX were determined using the corresponding regression equations, and the amount of added analyte concentrations was computed. The accuracy was expressed as percentage recovery and % RSD (Table 3). The percentage recovery was in the range of 98.70% to 101.83% with low % RSD (<2%), which indicates the best accuracy of the methods.

4.6. Stability Studies. Standard stock solutions and working standard solutions were stored in the refrigerator at 4°C and analyzed on a daily basis for 7 days. No variation in the concentration of both analytes was observed even on the 7th day, which indicates the stability of both analytes.

4.7. Application of Proposed Methods for Determination AMD and COX from the Formulation Mixture and Laboratory-

Prepared Tablets. Both UV spectroscopic procedures were effectively utilized for quantification of AMD and COX from the formulation mixture and laboratory prepared tablets (Supplementary material File (available here)). Fixed-dose combination of AMD and COX was not available in the local market; hence, separate formulations of AMD and COX were mixed in the proposition to get the concentration of coformulation, and tablets were prepared in laboratory and analyzed. The analysis results (Table 4) were in agreement with the amount of AMD and COX in the formulation. Further recovery studies results (Table 3) were also in agreement with the amount of AMD and COX added to the previously analyzed mixture. The validity of the methods was assessed by determining the accuracy of the methods by the standard addition method. The analysis results (Tables 3 and 4) with low % RSD showed the excellent accuracy and precision of the methods. In addition, it also proved the absence of excipients' interference in the analysis of analytes from formulations. Furthermore, the determined "t" and "F" values were less than the critical "t" and "F" values (Table 5), indicating that there is no significant difference in the analysis results between the two methods.

TABLE 4: Determination of AMD and COX from formulation mixture and tablets.

| | Formulation ratio (mg/Tab) | First derivative method | | Second method | |
| | | % Recovery | | % Recovery | |
		Formulation mixture	Tablets	Formulation mixture	Tablets
AMD	10	98.41	98.54	99.08	98.55
COX	200	99.29	101.87	98.90	99.03
AMD	5	99.21	101.08	100.36	100.89
COX	200	98.58	98.14	98.49	101.38
AMD	2.5	98.70	100.98		
COX	200	101.37	98.06		

TABLE 5: Statistical results of the comparison between the two proposed methods.

Parameters	AMD ($n = 6$)	COX ($n = 6$)
Average (% recovery)	99.58	100.09
% RSD	0.69	1.36
T (2.570)[a]	1.385	0.621
F (5.050)[b]	2.171	1.034

[a]The critical "t" value. [b]Critical "F" value at ($P = 0.05$).

5. Conclusions

Two simple UV spectroscopic procedures have been established for determination of AMD and COX in the presence of each other and excipients. These are the first analytical methods reported for determination of AMD and COX in the presence of each other. Both methods are simple, economical, eco-friendly, accurate, and precise. The first derivative spectroscopic method can determine both the analytes in a binary mixture in two steps: derivatization and measurement of amplitude at zero-crossing wavelength. On the other hand, the second method involves direct measurement of AMD and COX, which has been analyzed in three steps: division for ratio, derivatization, and measurement of amplitude; however, ratio derivatization gives many maxima and minima to determine the amount of analyte in the presence of another analyte. Finally, both the methods have been successfully applied for the determination of AMD and COX from formulation mixture and laboratory-prepared tablets. The statistical comparison results confirm that there is no significant difference between the two proposed methods.

Data Availability

The UV spectral data used to support the findings of this study are included within the article and supplementary information files.

Conflicts of Interest

The authors have no conflicts of interest.

Acknowledgments

Supplementary Materials

File 1: first derivative spectra of AMD and COX for precision and accuracy. File 2: (A) normal spectra of AMD and COX in a ratio 2 : 5, 4 : 20, and 8 : 40 μg/ml. Enlarged part for AMD (2, 4, 8 μg/ml) for measuring absorption at 363.4; (B) first derivative of ratio spectra of C5, 20, and 40 μg/ml for precision and accuracy. File 3: first derivative of Mixture of AMD and COX in a ratio 1 : 20 (formulation), 2 : 25, 3 : 30, and 4 : 35 μg/ml, respectively, for formulation and recovery studies. File 4: (A) ratio spectra of COX 20, 25, 30, and 35 μg/ml using AMD (2 μg/ml) solution spectrum as devisor; (B) first derivative of ratio spectra of COX 20, 25, 30, and 35 μg/ml using 4 nm as $\Delta\lambda$ for formulation and recovery studies. File 5: (A) normal spectra of AMD and COX in a ratio (a) 0.5 : 20, (b) 1 : 20, and (c) 0.5 : 40 μg/ml; (B) first derivative spectra of AMD and COX in a ratio (a) 0.5 : 20, (b) 1 : 20, and (c) 0.5 : 40 μg/ml; (C) (first method) ratio spectra of tablet solution of (a) COX 20 μg/ml and (b) COX 40 μg/ml using AMD (2 μg/ml) solution spectrum as devisor; (D) first derivative of ratio spectra of tablet solution of (a) COX 20 μg/ml and (b) COX 40 μg/ml using 4 nm as $\Delta\lambda$. (*Supplementary Materials*)

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