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Research Article

Green Simultaneous Determination of Amlodipine Besylate and Celecoxib by Dual wavelength and Simultaneous Equation Spectrophotometric Methods

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ABSTRACT

Two facile, accurate, green, and sensitive spectrophotometric methods; using dual-wavelength spectrophotometry (method I) and simultaneous equation method (method II), were developed for the simultaneous determination of amlodipine besylate (AMO) and celecoxib (CEX). Method I was based on measuring the absorbance difference between 252 nm and 357 nm for the determination of CEX, where AMO can be determined directly by measuring the absorbance at wavelength 357 nm as CEX has no absorbance at 357 nm. Method II was based on solving two simultaneous equations for both drugs at 252 nm and 357nm. The linearity range for AMO was found to be 5-65 µg/mL while for CEX was found to be 1-25 µg/mL for both methods. The developed methods were successfully applied for the determination of AMO and CEX in a laboratory prepared mixture containing all possible excipients present in a tablet dosage form. The mean percentage recovery values were found to be 101.157 ± 0.750, and 101.765 ± 0.140 for method I, but 101.603 ± 0.750, and 100.549 ± 1.262 for method II for AMO and CEX, respectively. The methods were found to be eco-friendly as they were evaluated according to the Green Analytical Procedure Index (GAPI) and Analytical Eco-Scale.

Keywords: Amlodipine besylate, Celecoxib, Dual wavelength, Analytical Eco-Scale.

1. INTRODUCTION

Amlodipine besylate (AMO; 3-Ethyl, 5-Methyl (±)-2-[(2-Aminoethoxy)methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydro-3,5-pyridinedicarboxylate Monobenzenesulfonate) is a calcium channel blocker.[1,2] It inhibits cardiac and vascular smooth muscle contraction through inhibition of calcium ions uptake into myocardial and vascular smooth muscle cells resulting in the reduction of coronary artery muscle tone and peripheral vascular resistance. AMO is clinically used for the treatment of hypertension, chronic stable angina, and prinzmetal's angina. AMO is official in the British Pharmacopoeia (BP),[1] the United States Pharmacopoeia (USP),[2] and the Indian Pharmacopoeia.[3]

Celecoxib (CEX; 4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl] benzenesulfonamide) is a non-steroidal anti-inflammatory drug that inhibits cyclooxygenase 2 enzyme (COX-2), resulting in decreasing tissue concentrations of prostaglandins to reduce pain. Prostaglandins sensitize afferent nerves and potentiate the action of bradykinin in inducing pain and are mediators of inflammation. Since CEX is an inhibitor of prostaglandin synthesis, it has analgesic, anti-inflammatory, and antipyretic

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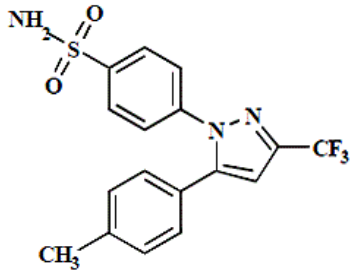
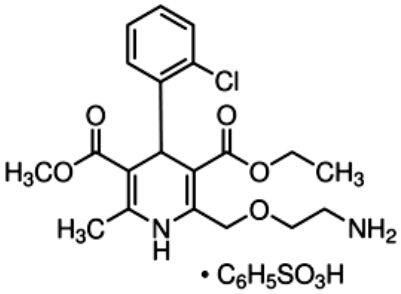
properties which are used for the treatment of the signs and the symptoms of osteoarthritis of the knee and hip.[4] CEX is official in both British Pharmacopoeia[1] and the United States Pharmacopoeia.[2] The chemical structures of AMO and CEX are shown in Figure 1.



(a) Celecoxib



(b) Amlodipine besylate

Figure 1: Chemical structure of (a) Celecoxib and (b) Amlodipine besylate.

Consensi®; is a newly FDA approved fixed-dose combination tablet dosage form of AMO and CEX at a ratio of 1:20, indicated for patients who have hypertension and osteoarthritis. It can be also used in lowering blood pressure and reducing the risk of fatal and non-fatal cardiovascular events, primarily strokes and myocardial infarctions.[5]

Several publications are describing analytical methods for the determination of AMO either alone or in combination with other drugs. These methods include capillary electrophoresis;[6,7] adsorptive square-wave anodic stripping voltammetry[8] and spectrophotometry with atorvastatin,9 or olmesartanmedoximil,[10] or valsartan and hydrochlorothiazide.[11] Different reverse phase high-performance liquid chromatography (RP-HPLC) methods have been developed for the determination of AMO in combination with other drugs such as metoprolol succinate,[12,13] benazepril hydrochloride,[14] and inhuman plasma.[15-17]

Different methods were developed for the determination of CEX including spectrophotometric methods either alone,[18] or with the interleukin-1 beta inhibitor diacerein using adsorption correction and chemometric method.[19] Different RP-HPLC methods have been developed for the determination of CEX in human plasma.[20-23] There is a spectrophotometric method for simultaneous determination of AMO and CEX has been reported,[24] which uses a solubilizing agent. A RP-HPLC method for simultaneous determination of AMO and CEX has been reported.[25]

The present work aims to develop two sensitive and fully validated spectrophotometric methods for simultaneous determination of AMO and CEX without prior separation. The methods were successfully used for the determination of both drugs in their bulk and in laboratory prepared tablets without interference from the excipients that may be present in dosage form. The proposed methods allow high sensitivity and increasingly fast analysis time. So, the developed methods have several advantages over other previously reported spectroscopic methods. These advantages make the developed methods suitable for routine quality control analysis.

2. Experimental

2.1. Apparatus and software

Spectrophotometric measurements were carried out using Shimadzu (UV-1800) UV-VIS double beam spectrophotometer equipped with 1 cm quartz cells and connected to a personal computer loaded with UV-Probe 2.33 software. Absorption spectra were recorded on a wavelength range of 200-400 nm at a scan rate of 400 nm/min and spectral bandwidth of 0.1 nm.

2.2. Material and reagent

AMO was kindly supplied by Sigma Company for Pharmaceutical Industries, Quesna, Menofia, Egypt. The purity was found to be 99.4%. CEX was kindly supplied by Amriya Pharmaceutical Industries, Alexandria, Egypt with a purity of 99.1%.

Acetonitrile was purchased from Sigma Aldrich, Germany. Mannitol DC 200, croscarmellose sodium, povidone K-30, sodium lauryl sulfate, magnesium stearate, and colloidal silicon dioxide were of analytical grade.

2.3. Stock and working solution

2.3.1. Stock standard solution

Stock standard solutions of AMO and CEX were prepared in acetonitrile to obtain solutions with a concentration of 1.0 mg/mL. AMO standard solution was protected from light due to its photosensitivity. The standard solutions were found to be stable for 10 days when kept in the refrigerator at 4^0C during this period the solutions remain suitable for their use in the analysis.

2.3.2. Working standard solution

Accurately measured volumes of stock standard solutions of AMO and CEX were transferred into two 10 mL volumetric flasks and diluted appropriately with acetonitrile to obtain

working standard solutions with concentration 100 µg/mL for AMO and CEX.

2.4. Construction of calibration curve

In a series of 10 mL volumetric flasks, different aliquots of the working standard solutions of AMO (100 µg/mL) and CEX (100 µg/mL) were transferred separately; several appropriate dilutions were carried out with acetonitrile to obtain solutions of AMO ranging from 5-65 µg/mL and solutions of CEX ranging from 1-25 µg/mL. UV spectra were scanned for these solutions using acetonitrile as blank.

2.4.1. Calibration curve of AMO at λ 357 nm

AMO was determined by measuring the absorbance at 357 nm. The calibration curve was obtained by plotting A (357nm) against corresponding concentrations for AMO.

2.4.2. Calibration curve of CEX by dual wavelength method

CEX was determined by measuring the ΔA between 252 nm and 357 nm; at which ΔA for AMO equals zero. The calibration curve was obtained by plotting ΔA (252nm-357nm) against corresponding concentrations for CEX.

2.4.3. Simultaneous equation spectrophotometric method

It was found that only AMO showed interference at λ_{max} of CEX, but CEX had no absorbance at λ_{max} of AMO. So, calibration curves were obtained by plotting the absorbance at λ 252 nm and 357 nm for AMO at λ 252 nm for CEX against their corresponding concentrations. Two simultaneous equations were constructed.

2.5. Preparation of laboratory prepared mixture

The dosage form Consensi® is not available in the local market, therefore a laboratory prepared mixture simulated to tablet dosage form was prepared by mixing of 10 mg AMO, 200 mg CEX, and the following excipients: 87 mg mannitol DC 200, 16.5 mg croscarmellose sodium, 6.6 mg povidone K-30, 3.3 mg sodium lauryl sulfate, 3.3 mg magnesium stearate and 3.3 mg colloidal silicon dioxide (aerosol).

The laboratory prepared mixture was transferred to a 100 mL volumetric flask, dissolved in 50 mL of acetonitrile, and sonicated for 15 minutes. Then, the solution was made up to the required volume using acetonitrile. The solution was filtered, and the first 10 mL of the filtrate was discarded. An aliquot equivalent to 1 mL of the filtrate was transferred to a 100 mL volumetric flask and made up to the final volume with acetonitrile. Different aliquots of this solution were transferred to a set of 10 mL volumetric flasks, spiked with 1 mL from a standard solution of AMO (100 µg/mL), and made up to final volume with acetonitrile to prepare solutions having concentrations within the linearity range of both drugs. The procedures were carried out as mentioned in section (2.4.) and then concentrations of both drugs were calculated from the corresponding regression equations of the two methods.

3. RESULTS AND DISCUSSION

The present work describes the development and validation of two simple, precise, and accurate spectrophotometric methods (dual-wavelength and simultaneous equation spectrophotometry) for the simultaneous determination of AMO and CEX in their combination at the dosage form ratio (1:20) for AMO and CEX, respectively. The methods were successfully applied to laboratory-prepared tablets.

3.1. Method development

The zero-order UV spectra of AMO and CEX **(Figure 2)** exhibits certain overlap, where AMO can be determined directly by measurement of the absorbance at λ 357 nm (A357nm), where CEX has no absorbance at 357 nm. CEX is difficult to be determined from a zero-order spectrum.

Dual-wavelength spectrophotometry offers an efficient method for analysing a component in presence of an interfering component. In this mixture, CEX is considered as a component of interest and AMO is considered as an interfering component. For elimination of interferences, two wavelengths were selected for CEX at the same time the difference in absorbance is zero for AMO.

3.1.1. Estimation of AMO at λ 357 nm

By overlaying the zero-order spectrum of AMO and CEX, it was found that AMO can be determined at 357 nm, as CEX has no absorbance at that wavelength. So, AMO can be determined directly from the zero-order spectrum. AMO was determined by measuring the absorbance at 357 nm.

3.1.2. Estimation of CEX by dual-wavelength method

By overlaying the zero-order spectrum of AMO and CEX, it was found that CEX cannot be determined directly in presence of AMO. For CEX, two wavelengths were selected at which the absorbance difference (ΔA) at these wavelengths equals zero for AMO. CEX was determined by measuring the ΔA between 252 nm and 357 nm; at which ΔA for AMO equals zero. AMO exhibits equal absorbance at 252 nm and 357 nm, then absorbance difference ΔA(252nm-357nm) for AMO equals zero. The measured ΔA(252nm-357nm) is concerned as a function of CEX concentration.

3.1.3. Simultaneous equation spectrophotometric Method

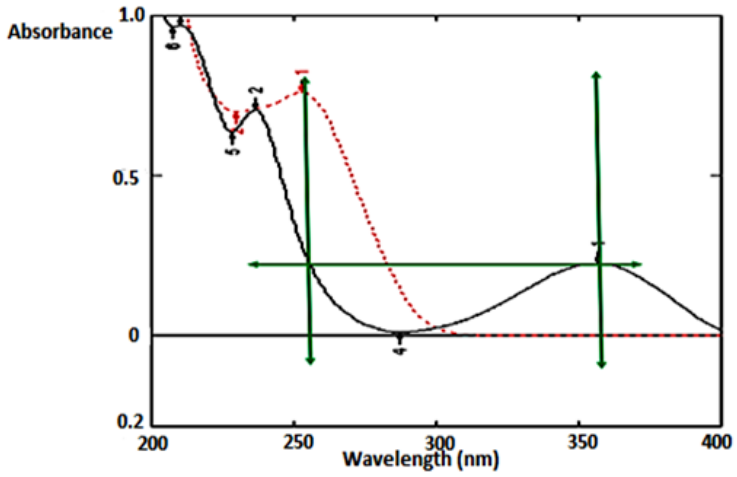


Figure 2. Zero-order UV spectra of 20 µg/mL celecoxib (……) and 15 µg/mL amlodipine besylate (___) in acetonitrile showing the selected wavelength for the dual-wavelength method.

In 1934, Vierordt's[26] developed an equation for the algebraic calculation of the individual absorbance of two components present in a mixture. This equation, which was later named after him, opened a wide field for the solution of spectrophotometric interference using many mathematical equations.

The UV absorbance of AMO and CEX were recorded within the wavelength range 200-400 nm at 0.1 nm interval. The wavelength of maximum absorbance (λ_{max}) was found to be 357 nm and 252 nm for AMO and CEX, respectively. The original zero-order spectra of AMO and CEX in acetonitrile showed a certain overlapping. λ_{max} values of AMO and CEX. Only AMO showed interference at the λ_{max} of CEX. Calibration curves were generated for AMO at 357 nm and 252 nm and CEX at 252 nm only.

The two simultaneous equations were generated as follows:

$A_{357} = a_{\underset{AMO}{357}}\, b\ C_{AMO}$ ……. (1)

$A_{252} = a_{\underset{CEX}{252}} b\ C_{CEX} + a_{\underset{AMO}{252}}\, b\ C_{AMO}$ ……. (2)

Where, $a_{\underset{AMO}{357}}$ and $a_{\underset{AMO}{252}}$ are the slope of calibration curve of AMO at 357 nm and 252 nm respectively, $a_{\underset{CEX}{252}}$ is the slope of the calibration curve of CEX at 252 nm.

3.2. Validation of the developed methods

The validity of the methods was studied regarding linearity, specificity, accuracy, and precision according to International Council for Harmonisation (ICH) guidelines.[27,28]

3.2.1. Linearity

Linearity was studied to determine the range over which analyte response is linear as a function of concentration. This study was performed by preparing standard solutions at seven different concentrations and analyses were performed in triplicates. The methods were found to be linear over a concentration range of 5-65 µg/mL for AMO and 1-25 µg/mL for CEX. Regression equations were calculated, the results of slope, intercept, standard deviation about the slope, and intercept. The quantitative statistical parameters for the determination of AMO and CEX are summarized in Table 1 The high values of correlation coefficients (r) with negligible intercepts indicate good linearity of the calibration curves.

3.2.2. Detection and quantitation limits

Table 1: Quantitative parameters for the determination of AMO and CEX by the proposed spectrophotometric methods.

Method	Drug	Linearity µg/mL	Λ (nm)	r^2	A	b	$S_{y/x}$	S_a	S_b (10^{-4})	DL µg/mL	QL µg/mL
Zero-order Method	**AMO**	5 – 65	357	0.9995	0.0005	0.0112	0.0053	0.00337	0.98	1	3
Dual Wavelength	**CEX**	1 – 25	252 – 357	0.9996	0.0018	0.0049	0.0094	0.00493	3.55	0.33	1
Simultaneous equation	**AMO**	5 – 65	357	0.9995	0.0005	0.0112	0.0053	0.00337	0.98	1	3
	CEX	1 – 25	252	0.9997	0.0032	0.0501	0.0075	0.00381	2.8	0.25	0.75

r2: coefficient of determination, a: intercept, b: slope, Sy/x: residual standard deviation of the regression line, Sa: standard error of intercept, Sb: standard error of slope, DL: detection limit (calculated), QL: quantitation limit (calculated).

Detection (DL) and quantitation (QL) limits can be calculated depending on the standard deviation of the response and the slope. They may be expressed as:

$DL = \frac{3.3\,\sigma}{S}$ and $QL = \frac{10\,\sigma}{S}$

where;"**σ**" is the standard deviation of the y-intercept of regression line (S_a) and "**S**" is the slope of the calibration curve.

For dual-wavelength method; calculated DL and QL were found to be 1 µg/mL and 3 µg/mL for AMO and 0.33 µg/mL and 1 µg/mL for CEX. DL and QL calculated using simultaneous equation method were 1 µg/mL and 3 µg/mL for AMO and 0.25 µg/mL and 0.75 µg/mL for CEX, respectively **(Table 1)**.

3.2.3. Accuracy

The accuracy of the proposed methods was evaluated by triplicate determination of three different concentrations of binary mixtures of AMO and CEX within the linearity range. Accuracy was expressed as the % recovery ± S.D.

Mean % recovery ± S.D was 99.082 ± 1.260 for AMO and 101.303 ± 0.620 for CEX, for the dual wavelength method. Mean % recovery ± S.D was 99.443 ± 1.280 for AMO and 99.687 ± 1.407 for CEX, for simultaneous equation method as shown in Table 2.

3.2.4. Precision

Precision was carried out for both methods by triplicate determination of three different concentrations of laboratory prepared mixture of AMO and CEX within the linearity range. Precision was carried out on the same day (intra-day precision) or in three successive days (inter-day precision). Standard deviation (S.D.) and % relative standard deviation (% R.S.D.) values of the results obtained were calculated.

The % R.S.D was not more than 0.285% for AMO and not more than 0.209% for CEX, for the dual-wavelength method. The % R.S.D was not more than 0.284% for AMO and not more than 0.182% for CEX, for simultaneous equation method as shown in Table 3.

3.2.5. Specificity

For testing the specificity of the proposed method; the % recovery of AMO and CEX was determined in laboratory prepared tablets containing both drugs with the possible excipients present in dosage form by the two proposed methods as shown in Table 4**.**

Mean % recovery ± S.D was 101.157 ± 0.750 for AMO and 101.765 ± 0.140 for CEX, for the dual-wavelength method. Mean % recovery ± S.D was 101.603 ± 0.750 for AMO and 100.549 ± 1.262 for CEX, for simultaneous equation method. This confirmed that the excipients in the laboratory prepared tablets did not show any interference.

3.2.6. Assessment of the greenness of the proposed methods using green analytical procedure index (GAPI) and analytical eco-scale

Analytical eco-scale is a new semi-quantitative mean for evaluation of analytical practice and educational attributes in green analytical chemistry protocols. Even though it compares different steps and parameters in the analytical practice, it does not supply us with comprehensive data about evaluation protocols.[29,30] The ideal green analysis has an analytical Eco-Scale value of 100 and the penalty points of (energy, reagent, waste, and hazard) were subtracted from the ideal green analysis. According to that, the developed spectrophotometric methods were found to have an analytical Eco-Scale value of 84 indicating an excellent green method of analysis with low laboratory requirements as shown in Table 5.

Green Analytical Procedure Index (GAPI) indicates the green properties of all over the analytical practice, from sampling to sample preparation, storage, transport, and final determination GAPI has the advantage that it supplies data

Table 2: Evaluation of the accuracy for the determination of AMO and CEX by the proposed spectrophotometric methods.

Concentration taken (µg/mL)		Dual wavelength						Simultaneous equation					
		Concentration found (µg/mL)			Mean concentration found (µg/mL)	% Recovery	Mean % Recovery ± S.D.	Concentration found (µg/mL)			Mean concen-tration found (µg/mL)	% Recovery	Mean % Recovery ± S.D.
AMO	10.8	10.750	11.000	10.804	10.852	100.474	99.082 ± 1.260	10.795	11.045	10.848	10.896	100.887	99.443 ± 1.280
	10	9.830	9.794	9.777	9.801	98.006		9.875	9.839	9.821	9.845	98.452	
	20	19.929	19.429	19.902	19.753	98.765		19.973	19.473	19.946	19.798	98.988	
CEX	16	16.142	16.478	16.281	16.300	101.877	101.303 ± 0.620	16.248	16.166	16.200	16.205	101.279	99.687 ± 1.407
	10	10.148	10.126	10.142	10.138	101.383		9.854	9.849	9.879	9.861	98.607	
	20	20.372	20.012	20.004	20.130	100.648		19.701	20.040	19.765	19.835	99.176	

Table 3: Evaluation of the intra-day and inter-day precision for the determination of AMO and CEX by the spectrophotometric methods

		Dual wavelength						Simultaneous equation					
		Intra-day			Inter-day			Intra-day			Inter-day		
Concentration taken (µg/mL)		Mean concent-ration found* (µg/mL)	% Recov-ery	% R.S.D	Mean concentr-ation found* (µg/mL)	% Recov-ery	% R.S.D	Mean concentra-tion found* (µg/mL)	% Recov-ery	% R.S.D	Mean concent-ration found* (µg/mL)	% Reco-very	% R.S.D.
AMO	10.8	10.851	100.474	0.131	10.741	99.454	0.097	10.896	100.887	0.130	10.786	99.868	0.096
	10	9.801	98.006	0.028	9.823	98.234	0.022	9.845	98.452	0.028	9.879	98.795	0.015
	20	19.753	98.765	0.285	19.815	99.077	0.078	19.798	98.988	0.284	19.860	99.301	0.078
CEX	16	16.300	101.876	0.166	16.232	101.448	0.080	16.205	101.279	0.041	16.182	101.137	0.020
	10	10.138	101.383	0.011	10.121	101.215	0.050	9.861	98.607	0.016	9.906	99.062	0.088
	20	20.130	100.648	0.209	20.140	100.700	0.063	19.835	99.176	0.182	19.880	99.402	0.065

about quantification of the evaluated procedure.[31] GAPI has five pentagrams which are used to evaluates the environmental effect on each step of the proposed method, through three colors: red, yellow, and green representing high, medium to low effects, respectively. The additional information obtained from GAPI pictograms is about the extraction process is included, and if it is included which type of extraction and its scale.

According to the GAPI pictograms, the same results obtained from Analytical Eco-Scale analysis were determined, as shown in Figure 3, the spectrophotometric methods are direct green methods, without any extraction procedures, and the use of small volume from non-toxic solvents. The waste volume is low. Also, this method is for quantification and qualification.

3.2.7. Comparison with other reported methods:

Data presented in Table 6 compares results obtained using the methods hereby proposed with other methods already described in literature.[24, 25] This comparison reveals that the proposed methods show a fast method that doesn't need a long time in manipulation and pre-treatment of the sample preparation as in the reported spectroscopic and HPLC methods. It is used eco-friendly reagents and solvents without need of tedious procedure. So, the proposed method has the lowest impact on the environment according to the used reagents and compounds. Moreover, in terms of instrumentation and waste, it has lower environmental impact than the reported methods.

Table 4: Recovery data of Amlodipine besylate and Celecoxib from laboratory prepared tablets by the proposed spectrophotometric methods

		Dual wavelength						Simultaneous equation					
Concentration taken(µg/mL)		Concentration found (µg/mL)			Mean concentration found(µg/mL)	% Recovery	Mean % Recovery ± S.D.	Concentration found (µg/mL)			Mean concentration found(µg/mL)	% Recovery	Mean % Recovery ± S.D.
AMO	**0.4**	0.405	0.407	0.408	0.407	101.625	**101.157**	0.407	0.409	0.409	0.408	102.071	**101.603**
	0.8	0.811	0.812	0.815	0.812	101.554	±	0.815	0.815	0.818	0.816	102.000	±
	1.2	1.196	1.206	1.208	1.204	100.292	**0.75**	1.202	1.211	1.214	1.209	100.738	**0.75**
CEX	**8**	8.087	8.176	8.178	8.146	101.835	**101.765**	7.896	7.955	7.961	7.938	99.219	**100.549**
	16	16.277	16.301	16.313	16.297	101.856	±	16.123	16.132	16.080	16.112	100.699	±
	24	24.380	24.347	24.428	24.385	101.605	**0.140**	24.292	24.491	24.462	24.415	101.730	**1.262**

Table 5: The penalty points of the proposed methods according to the analytical Eco-Scale per sample

Reagents	**Penalty points**
Acetonitrile	8
Instrument	**Penalty points**
UV (≤ 1.5 kWh per sample)	0
Occupational hazard (analytical process hermitization)	0
Waste (<10 mL, no treatment)	8
Total penalty points	**16**
Analytical eco-scale total score [a]	**84**

aIf the score is > 75, it represents excellent green analysis
If the score is > 50, it represents acceptable green analysis
If the score is <50, it represents inadequate green analysis

Table 6: Comparison of the analytical parameters among the proposed and the reported methods

Parameters	**Proposed spectroscopic methods**		**HPLC method**		**Reported spectroscopic method**	
	AMO	**CEX**	**AMO**	**CEX**	**AMO**	**CEX**
Linearity range (µg/ml	5 – 65	1-25	5-30	50-500	2-10	10-50
Mobile phase or diluting solvents used	Acetonitrile		Phosphate buffer (20mM, PH adjusted to 5.6 with diluted NaOH): acetonitrile and methanol at a ratio 30:55:15(v/v) Eluted at 1.2 mL/min		2 M sodium benzoate as hydrotropic solubilizing agent	
Analytical Eco-Scale value	84		77		84	
Analysis time(min) (including sample pre-treatment)	18		21		1440	

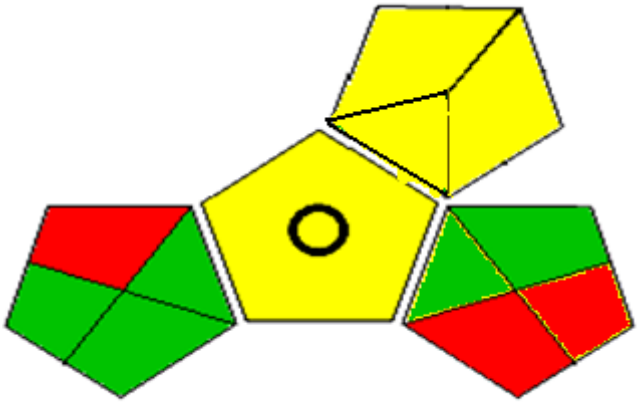


Figure 3: The Green Analytical Procedure Index profile for the proposed spectrophotometric methods.

4. CONCLUSIONS

The developed methods are simple, accurate, robust, sensitive, and green for the simultaneous estimation of celecoxib and amlodipine besylate in the ratio (20: 1) as in their pharmaceutical dosage forms. The excipients present in tablets dosage form did not interfere in the analysis, which proved the specificity of the method for these drugs. The sample preparation is simple and rapid. Hence, the proposed spectrophotometric methods can be used for the routine quality control analysis in the combined formulation either in authentic samples or in dosage forms, and they can be applied to the toxicological and biological work.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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