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Research article

Two eco-friendly spectrophotometric methods for the concurrent estimation of diacerein and meloxicam: greenness and whiteness assessment

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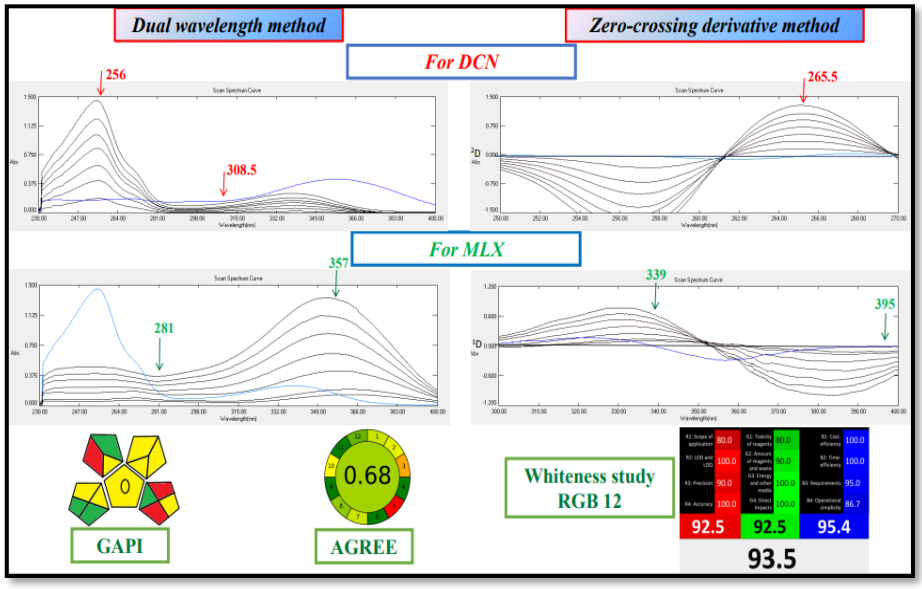
Abstract:



The current study represents the concurrent analysis of diacerein and meloxicam in their binary mixture by two simple spectrophotometric methods, for the first time without prior separation, namely the dual-wavelength and the zero-crossing derivative spectrophotometry methods. Firstly, the dual wavelength method involves the selection of two wavelengths at the original absorption spectra for each drug in such a way that the subtraction of absorbance readings equals zero for the second drug. Wavelengths at 256.0 and 308.5 nm were chosen for the estimation of diacerein where meloxicam showed equal absorption values. Similarly, wavelengths at 357.0 and 281.0 nm were selected for the determination of meloxicam without any contribution of diacerein. Secondly, the other method depends on derivative spectrophotometry with zero-crossing measurement to solve the spectral overlap problem where diacerein was easily assayed using ^{2}D amplitudes at 265.5 nm with no contribution from meloxicam.

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Also, meloxicam was determined by calculating the sum of the absolute values of ^{1}D amplitudes at 339 and 395 nm, where diacerein was zero-crossing at these wavelengths. The ICH guidelines were used to validate the analytical performance of the adopted methods. For the two adopted methods, diacerein and meloxicam showed perfect linearities in the ranges 2-14 µg/mL and 3-24 µg/mL, respectively, with high correlation coefficients (≥ 0.99975). The validated procedures were effectively used in the concurrent quantification of both drugs either in bulk powders, synthetic mixtures, or laboratory-prepared capsules. Finally, the greenness and whiteness of the adopted methods were assessed and compared among other reported procedures.

Keywords: Spectrophotometric determination; Diacerein; Meloxicam; Greenness and whiteness assessment; Combined capsules.

1. Introduction

Osteoarthritis (OA) is a life-lasting skeletal disease recognized by increasing deterioration and erosion of cartilage together with the generation of osteophytes leading to inflammation of the synovial membrane [1,2]. Because of the intricate and multifaceted symptoms of OA, drug combination therapy is frequently favorable to prevent disease progression as well as to manage the associated pain [3,4].

Non-steroidal anti-inflammatory drugs (NSAIDs), such as Meloxicam (MLX) (4-Hydroxy-2-methyl-N-(5-methylthiazol-2-yl)–2H-1,2-benzo-thiazine-3-carboxamide-1,1-dioxide) (**Fig. 1)** are the first choice for the alleviation of OA-generated pain [2]. MLX is an oxicam derivative characterized by possessing selective inhibition of COX-2 enzyme with powerful anti-inflammatory and analgesic actions due to inhibition of prostaglandins formation [5]. However, we cannot rely solely on NSAIDs in the treatment of OA as they possess several adverse effects and do not play a role in the recovery or delayed progression of OA. Therefore, Diacerein (DCN) (4,5-Diacetoxy-9,10-dioxo-9,10-dihydroanthracene-2-carboxylic acid) (**Fig. 1)**, is a disease-modifying OA drug (DMOADs) or chondroprotective agent [6]. DCN inhibits the production and activity of the pro-inflammatory cytokine Interleukin-1 beta (IL-1β), so it delays all pathological processes generated by OA [7]. Moreover, DCN increases the formation of hyaluronic acid and proteoglycans that maintain cartilage integrity. For the treatment and management of OA, combining DCN with NSAIDs (such as MLX) [3] provides the advantage of accelerating the response in the treatment of OA by simultaneously decreasing pain and improving joint integrity.

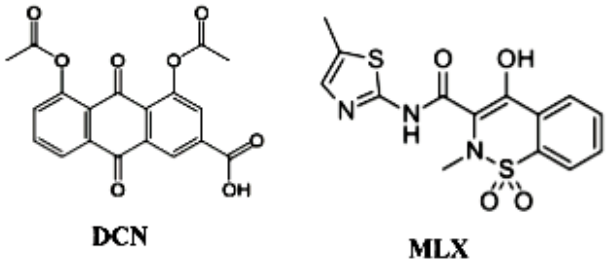

Fig. 1: Chemical structures of Diacerein (DCN) and Meloxicam (MLX).

Generally, the synchronized spectrophotometric determination of several components with overlapping spectra without preliminary separation is one of the major challenges encountered by chemical analysts. Two spectrophotometric methods were applied in this study to resolve the issue of overlapped spectra namely the dual wavelength and zero-crossing methods. The dual wavelength method was applied in several articles for the quantification of binary mixtures without previous separation [8-10]. For a binary mixture of two analytes X

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and Y, the dual wavelength method depends on the selection of two wavelengths where component Y shows the same absorbance while component X shows a significant difference in absorbance. Thus, the absorbance differences at these two points are directly proportional to the concentrations of component X, independent of component Y. Regarding the zero-crossing method, it is the most widely used technique in the estimation of drug mixtures [11,12]. It is a simple method based on derivative spectrophotometry where one analyte can be estimated at a selected wavelength, ideally peak or trough where the other was zero-crossing at this point.

A careful survey of the scientific database was performed, and a few published papers tackle the concurrent quantification of DCN in its binary mixture with MLX. Stability indicating HPLC procedure was published for the concurrent quantification of DCN and MLX[13], Recent multi-analyte HPTLC and HPLC- DAD methods were reported on the simultaneous quantification of DCN, MLX, and other NSAIDs [14,15]. The scientific literature lacks any published paper tackling the simultaneous spectrophotometric determination of DCN and MLX. Generally, the optical techniques and the UV-spectroscopy specifically represent the most appropriate techniques applied for routine analysis of active pharmaceutical ingredients due to their several pros such as simplicity, cost-effectiveness as well as their widespread availability in almost all quality control laboratories. Accordingly, this has motivated us to develop two simple and effective spectrophotometric methods for the synchronized estimation of DCN and MLX.

2. Experimental

2.1. Instrumentation

A T80 double-beam UV-Vis spectrophotometer (PG Instruments Ltd, London, United Kingdom), was used, linked to a personal computer (PC) loaded with UVWin 5 software (Version 5.2.0) using a pair of 1 cm matched quartz cells.

2.2. Materials and reagents

DCN was donated by Pfizer Pharmaceuticals, Dokki, Giza, Egypt. MLX was gifted by Amriya Pharmaceutical Industries, Alexandria, Egypt. HPLC grade methanol and acetonitrile (Fisher Scientific, Loughborough, UK) and dimethyl sulphoxide (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) were used during the study. The formulation analyzed in the current work was prepared in the laboratory in the form of capsules' powder comprising DCN and MLX. Inactive ingredients for laboratory preparations of dosage forms including starch, microcrystalline cellulose, magnesium stearate, and silica (El-Nasr Pharmaceutical Chemicals Co., Qaliubiya, Egypt) were also used.

2.3. Preparation of stock and working standard solutions

DCN stock solution (100 µg/mL) and MLX stock solution (200 µg/mL) were prepared in 2% dimethyl sulphoxide in acetonitrile (DMSO/ACN). Different accurate volumes of DCN and MLX stock solutions were further diluted in a series of 10 mL volumetric flasks, volumes were set to 2 mL with 2% (DMSO/ACN) and then completed with methanol to finally reach concentration ranges of 2-14 and 3-24 µg/mL for DCN and MLX, respectively.

2.4. Construction of the calibration graphs

The absorption spectra of the prepared serially diluted solutions were recorded and saved in the range of 230–400 nm.

Method I: Dual wavelength spectrophotometry

For DCN, the absorption difference of the zero-order spectra at 256.0 and 308.5 nm (the difference is zero for MLX) was plotted versus its corresponding concentrations. Similarly, for MLX determination, a

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calibration graph was plotted connecting the difference in absorbance of the zero-order spectra of MLX at 357.0 and 281.0 nm (the difference is zero for DCN) versus its corresponding concentrations. Then, regression equations were calculated (**Fig. 2, 3 and 4**).

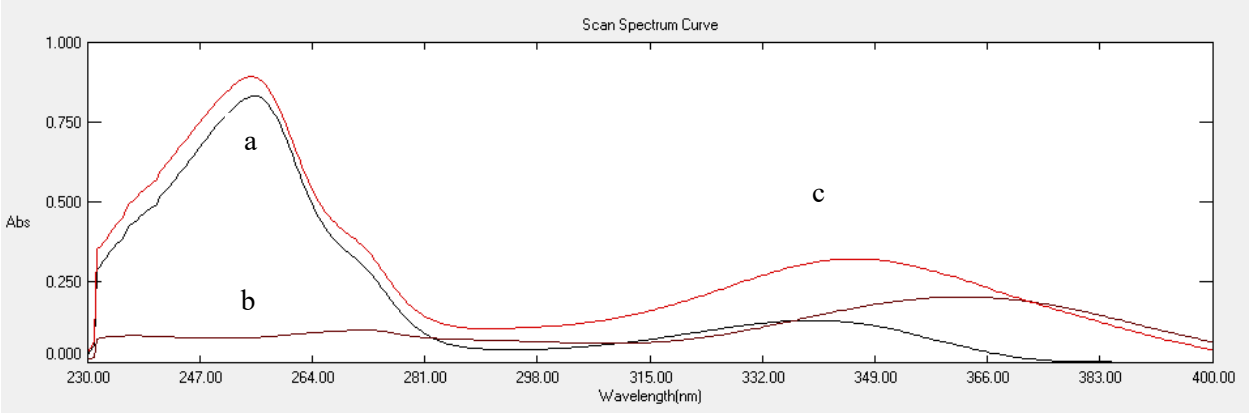

Fig. 2: Absorption spectra of 8 µg/mL DCN (a), 4 µg/mL MLX (b), and a mixture of 8 µg/mL DCN and 4 µg/mL MLX in methanol (c).

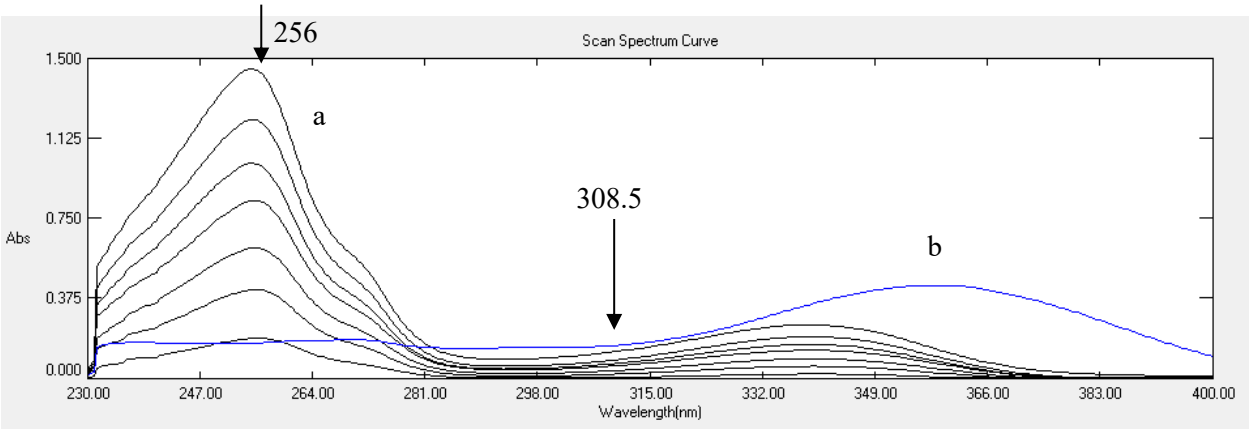

Fig. 3: Zero order spectra of 2, 4, 6, 8, 10, 12, and 14 µg/mL DCN (a) and 8 µg/mL MLX (b) in methanol.

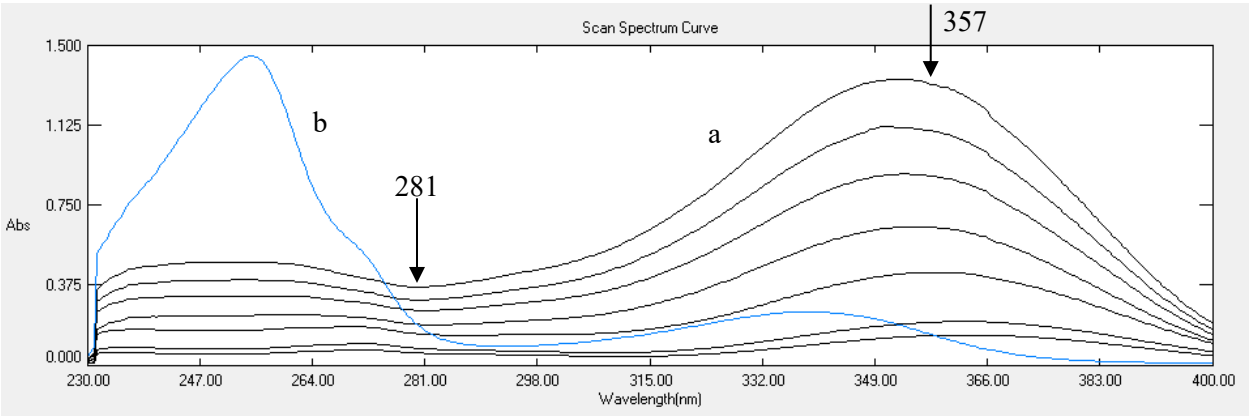

Fig. 4: Zero order spectra of 3, 4, 8, 12, 16, 20, and 24 µg/mL MLX (a) and 14 µg/mL DCN (b) in methanol.

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Method II: zero-crossing derivative spectrophotometry

For DCN determination, the second-order derivative (^{2}D) spectra (Δλ = 9) with scaling factor of 60 were recorded against the blank solution. Measurement of the ^{2}D amplitudes at 265.5 nm was performed for DCN. Likewise, for MLX, The first-order derivative (^{1}D) spectra (Δλ = 9) with scaling factor 30 were recorded against the blank solution. The sum of the absolute values of ^{1}D amplitudes at 339 and 395 nm was measured for MLX assay. Then, the recorded values of DCN and MLX were plotted versus their corresponding concentrations, and then calculating the regression equations (**Fig. 5 and 6**).

2.5. Assay of laboratory-prepared mixtures

The preparation of solutions of synthetic mixtures was carried out by mixing accurate volumes from the standard solutions of DCN and MLX at various ratios, both greater and lower than the marketed ratio in the formulation, into 10 mL volumetric flasks. Volumes were set to 2 mL with 2% (DMSO/ACN), then completed with methanol, and analysis was performed following the proposed spectrophotometric methods as under "Construction of the calibration graphs".

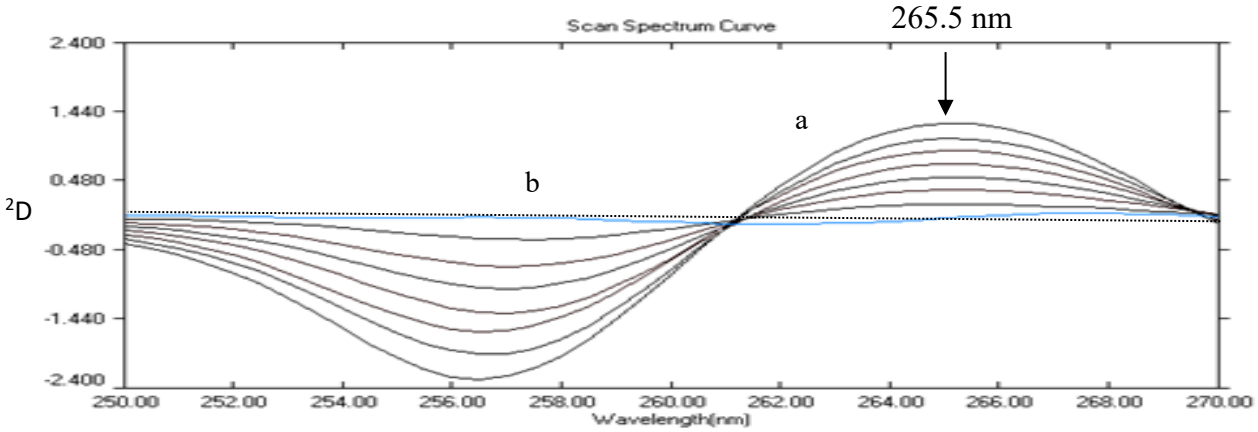

Fig. 5: Second-order spectra of 2, 4, 6, 8, 10, 12 and 14 µg/mL DCN (a) and 24 µg/mL MLX (b) in methanol.

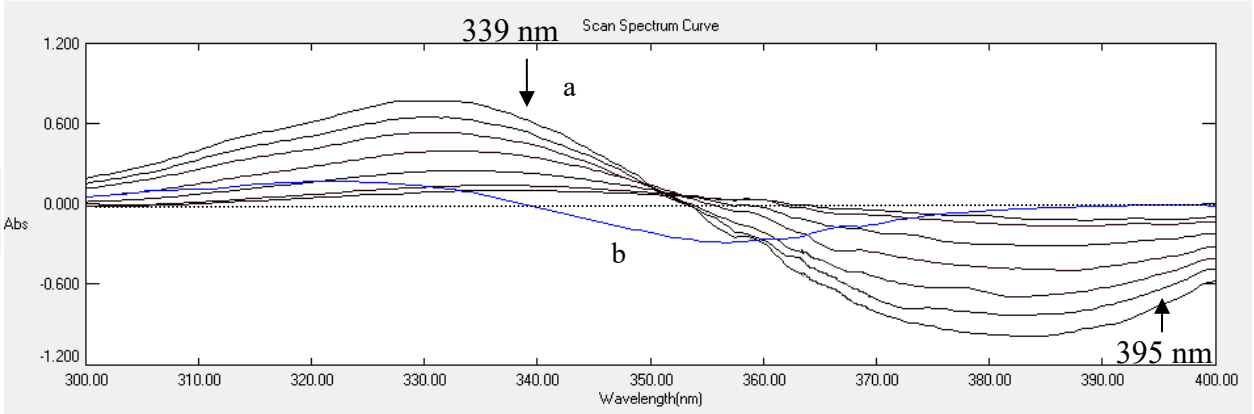

Fig. 6: First-order spectra of 3, 4, 8, 12, 16, 20 and 24 µg/mL MLX (a) and 14 µg/mL DCN (b) in methanol.

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2.6. Assay of DCN-MLX laboratory-prepared capsules

In the laboratory, we have prepared a powder mimicking that contained in the marketed Dolocartigen® capsules (each capsule encloses 50 mg DCN and 15 mg MLX). Additionally, the inactive components were added, such as starch, microcrystalline cellulose, magnesium stearate, and silica, in similar quantities as the marketed capsules. The powdered content of ten capsules was weighed. The accurately weighed portion of the powder similar to the average weight of one capsule powder was delivered to a 100 mL volumetric flask. A volume of 50 mL of 2% (DMSO/ACN) was added, followed by sonication for 20 min and filtration into a 100 mL volumetric flask. Washing of the residue was done triple each time using 10 mL of the same solvent. Collection of washings was done and then delivered to the filtered solution and the volume was then made up by the same solvent to attain a concentration of 150 µg/mL for MLX (MLX stock sample solution). A 10 mL aliquot of the prepared solution was delivered to a 50 mL volumetric flask and diluted with the same solvent to get a concentration of 100 µg/mL for DCN (stock sample solution of DCN).

Measured volumes of the two prepared stock sample solutions were accurately delivered to 10 mL volumetric flasks, continued to 2 mL with 2% (DMSO/ACN), and then completed with methanol to obtain the specified concentrations. The working sample solutions were then treated as under "Construction of the calibration graphs". Calculation of the percentage recoveries was carried out using concurrently prepared standard solutions (external standard method). Also, the standard addition method was carried out by accurate addition of various volumes of standard solutions of each analyte to its sample solutions to reach final concentrations among its specified linearity ranges then analyzed as previously described. The found concentrations were calculated by comparison of the rise in response attained by the standard solution added.

3. Results and discussion

3.1. Absorption characteristics

Spectrophotometry is regarded as a simple, accurate, and cost-effective tool that is always developed and applied in analytical chemistry and is specifically favored in quality control units. The zero-order absorption spectra of DCN and MLX, represented in **Fig. 2,** are found to be strongly overlapped. This overlapping prevents the employment of ordinary UV spectrophotometry for the simultaneous estimation of DCN and MLX unaccompanied by data manipulation. Accordingly, this necessitates the development of spectrophotometric methods to resolve this interference for the concurrent estimation of DCN and MLX without the need for separation. In the presented work, selective and accurate determination of both analytes could be achieved by applying two methods named dual wavelength and zero-crossing derivative spectrophotometric methods. These methods resolve the problem of overlapped spectra in addition to the advantages of minimal sample and data manipulation.

Method I: Dual wavelength spectrophotometry

This procedure utilizes the original zero-order absorption spectrum of the mixture and the issue of overlapping was fixed directly with no changes or manipulation [8]. The principle of the method depends on selecting two wavelengths where one drug possesses a significant difference in absorbance while the other combined drug possesses equal absorbance readings at these wavelengths. Hence, the absorbance difference (ΔA) at the two chosen wavelengths can be correlated to the concentrations of the former drug.

Optimization of the selected wavelengths is of great importance; therefore, different sets

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of wavelengths were tested such as 255.0, 245.0 nm and 256.0, 308.5 nm for DCN assay and 357.0, 281.0 nm and 357.0, 321.0 nm for MLX estimation. Wavelengths of 256.0 and 308.5 nm were chosen for the quantification of DCN as they resulted in excellent recoveries of different synthetic mixtures. **Fig. 3** shows the difference in DCN absorbance values at 256.0 and 308.5 nm where MLX signified equal absorption values. Similarly, using 357.0 and 281.0 nm gave the best results for the determination of MLX where the contribution of DCN was canceled due to identical absorbance values (**Fig. 4**).

Method II: zero-crossing derivative spectrophotometry:

Another simple method was developed based on derivative spectrophotometry to solve the problem of spectral overlap. The zero-crossing technique is the most common derivative spectrophotometric measurement. The effect of Δλ and scaling factor was carefully tested to optimize the position of peaks with respect to zero-crossing points and their amplitudes, respectively. In this binary mixture, Δλ = 9 nm was optimal for the determination of both analytes. The second (^{2}D) derivative spectra with scaling factor 60 enabled DCN analysis at 265.5 nm with no contribution from MLX (**Fig. 5**). Also, the first (^{1}D) derivative signals (scaling factor 30) permitted the determination of MLX at 339 and 395 nm where DCN was zero-crossing at these wavelengths (**Fig. 6**). The sum of the absolute values of ^{1}D amplitudes at 339 and 395 nm was used for the assay of MLX to maximize the amplitudes, thus improving sensitivity.

3.2. Validation of the proposed methods:

The criteria of the validation were accomplished based on the International Council for Harmonization recommendations for the validation of analytical procedures [16].

3.2.1. Linearity and concentration ranges

The linearity of the drugs under investigation was assessed by the analysis of seven concentrations of DCN and MLX. Then, least-squares treatment was used to construct linear regression equations of the measured responses of each drug versus their respective concentrations. **Table 1** demonstrates linearity data for the two methods. DCN and MLX showed perfect linearity within their concentration ranges with high correlation coefficients (≥ 0.99975) and low RSD% of the slopes (≤ 1.00).

Table 1: Analytical parameters for the determination of DCN and MLX using the proposed spectrophotometric methods.

Parameters	Method I		Method II	
	DCN	MLX	DCN	MLX
Wavelength (nm)	(256-308.5)	(357-281)	^{2}D $_{265.5}$	^{1}D $_{(339+395)}$
Linearity range (µg/mL)	2-14	3-24	2-14	3-24
Intercept (a)	0.0120	-0.0435	-0.0169	0.0522
Slope (b)	0.0942	0.0421	0.0919	0.0561
Correlation coefficient(r)	0.99976	0.99989	0.99977	0.99985
S_a	0.0082	0.0040	0.0078	0.0062
S_b	0.0009	0.0003	0.0009	0.0004
RSD% of slope (S_b%)	0.96	0.71	0.98	0.71
$S_{y/x}$	0.0097	0.0054	0.0093	0.0084
LOD (µg/mL)	0.34	0.42	0.33	0.49
LOQ (µg/mL)	1.03	1.28	1.01	1.50

S_a Standard deviation of the intercept
S_b Standard deviation of the Slope
$S_{y/x}$ Standard deviation of the residuals
LOD Limit of detection
LOQ Limit of quantification

3.2.2. Limits of detection and quantification

Using the ICH guidelines, the limits of detection (LOD) and limits of quantification (LOQ) were computed via the residual standard deviation of the regression line (σ) and the slope of the calibration curve (s) as follows: LOD = 3.3 (σ/s) and LOQ =10 (σ /s) then illustrated in **Table 1**. The obtained

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values verify the developed methods' sensitivity.

3.2.3. Accuracy and precision

The accuracy and intra-day precision (repeatability) for the suggested methods were checked by applying the proposed methods in performing triple determinations of each of the three concentrations among the linearity of each analyte in a single day. Also, the accuracy and inter-day precision (intermediate precision) were confirmed by the analysis of the same concentrations triple times on three different days. The concentrations were attained from the corresponding regression equations computed for each method and then percentage relative standard deviation (RSD %) and percentage relative error (E_r %) were computed and presented in **Table 2**.

The acceptable level of precision and accuracy of the adopted procedures was ascertained by the small RSD % and E_r % values (≤ 2%).

3.2.4. Selectivity

Various synthetic mixtures were analyzed using the proposed methods to assess the methods' selectivity. These mixtures were prepared at various ratios of pure DCN and MLX both higher and lower than the marketed ratio in combined pharmaceutical formulation. Satisfactory results of recovered concentrations, RSD% and E_r% were obtained and demonstrated in **Table 3**. The results proved the successful selectivity of the established spectrophotometric procedures in determining both drugs in the presence of each other and at varying concentration ratios.

Table 2: Accuracy and precision for the analysis of DCN and MLX in bulk form using the proposed spectrophotometric methods.

		Parameter	Concentration (µg/mL)	Found ± SD[a] (µg/mL)	RSD(%)[b]	E_r(%)[c]
Method I	**DCN**	**Intra-Day Precision**	2	1.97 ± 0.02	1.02	-1.50
			6	6.06 ± 0.03	0.50	1.00
			12	12.09 ± 0.10	0.83	0.75
		Inter-Day Precision	2	1.98 ± 0.03	1.52	-1.00
			6	6.09 ± 0.08	1.31	1.50
			12	12.12 ± 0.11	0.91	1.00
	MLX	**Intra-Day Precision**	4	3.96 ± 0.04	1.01	-1.00
			12	12.04 ± 0.11	1.00	0.33
			24	23.86 ± 0.04	0.17	-0.58
		Inter-Day Precision	4	3.99 ± 0.07	1.75	-0.25
			12	12.17 ± 0.14	1.15	1.42
			24	24.41 ± 0.46	1.88	1.71
Method II	**DCN**	**Intra-Day Precision**	2	1.99 ± 0.03	1.51	-0.50
			6	5.88 ± 0.06	1.02	-2.00
			12	11.82 ± 0.02	0.17	-1.50
		Inter-Day Precision	2	1.97 ± 0.03	1.52	-1.50
			6	6.05 ± 0.09	1.49	0.83
			12	12.22 ± 0.09	0.74	1.83
	MLX	**Intra-Day Precision**	4	4.02 ± 0.05	1.24	0.50
			12	11.88 ± 0.05	0.42	-1.00
			24	23.92 ± 0.21	0.88	-0.33
		Inter-Day Precision	4	3.97 ± 0.06	1.51	-0.75
			12	12.09 ± 0.14	1.16	0.75
			24	24.02 ± 0.35	1.46	0.08

[a] Mean ± standard deviation for three determinations.
[b] % Relative standard deviation., [c] % Relative error.

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Table 3: Determination of DCN-MLX laboratory-prepared mixtures using the proposed spectrophotometric methods.

	Concentration (µg/mL)		DCN			MLX		
Method I	**DCN**	**MLX**	**Found ± SD[a] (µg/mL)**	**RSD%[b]**	**E_r (%)[c]**	**Found ± SD[a] (µg/mL)**	**RSD%[b]**	**E_r (%)[c]**
	8	**4**	7.94 ± 0.05	0.63	-0.75	4.07 ± 0.05	1.23	1.75
	12	**4**	11.87 ± 0.12	1.01	-1.08	4.05 ± 0.06	1.48	1.25
	12	**3**	12.05 ± 0.25	1.99	0.42	3.04 ± 0.05	1.64	1.33
	Concentration (µg/mL)		**DCN**			**MLX**		
Method II	**DCN**	**MLX**	**Found ± SD[a] (µg/mL)**	**RSD%[b]**	**Er (%)[c]**	**Found ± SD[a] (µg/mL)**	**RSD%[b]**	**Er (%)[c]**
	8	**4**	8.14 ± 0.05	0.61	1.75	3.97 ± 0.06	1.51	-0.75
	12	**4**	12.21 ± 0.09	0.74	1.75	3.98 ± 0.05	1.26	-0.50
	12	**3**	12.08 ± 0.20	1.66	0.67	3.04 ± 0.03	0.99	1.33

[a] Mean ± standard deviation for three determinations.
[b] % Relative standard deviation.
[c] % Relative error.

3.3. Stability of solutions

To assess the stability of standard working solutions in methanol during analysis time, these solutions were analyzed every 30 min for 2 h upon storing at room temperature. The results revealed that no considerable changes in the absorption characteristics were found within 2 hr. Also, the stock standard solutions in 2% (DMSO/ACN) were found stable for 3 days upon storage in the refrigerator at 4 ºC.

3.4. Analysis of pharmaceutical formulation

The adopted procedures were carried out for the estimation of DCN and MLX in their laboratory-made formulation. No interference was observed from the formulation matrix. **Table 4** shows the analysis results of DCN and MLX in their formulation using external standard and standard addition methods. Excellent accuracy and precision were ascertained by the found concentrations, SD, and RSD% values (≤ 1.8). Therefore, the adopted procedures are suitable for the routine determination of these analytes in their combined formulation with reasonable selectivity, accuracy, and precision.

Furthermore, by the one-way analysis of variance test (Single factor ANOVA), the results of the adopted spectrophotometric procedures and the previously reported one [13] were statistically compared. The ANOVA test was performed for comparison of data obtained from the three methods[17]. It was found that the calculated F-values were lower than the tabulated value, verifying no pronounced differences between different adopted and reference methods **(Table 4).** The adopted and reference procedures are successfully appropriate to the concurrent quantification of DCN and MLX in their pharmaceutical formulation with good and analogous analytical performance.

3.5. Appraisal of the established methods' greenness and comparison with published ones

Recently, green analytical chemistry (GAC) has vast attention, moreover, a growing number of informative tools have been developed to appraise the environmental effect and safety of different analytical procedures. In this work, a detailed greenness evaluation was accomplished to compare the suggested spectrophotometric methods with published separation techniques for the

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concurrent quantitation of DCN and MLX[13-15]. The application of the green analytical procedure index (GAPI) [18] and the Analytical GREEnness calculator (AGREE) [19]

Table 4: Application of the proposed spectrophotometric procedures to the analysis of DCN-MLX mixture in its laboratory-prepared capsules using external standard and standard addition methods.

Parameters/Method		%Recovery ± SD[a]	RSD%[b]
External standard method			
DCN	**Method I**	100.06 ± 1.24	1.24
	Method II	99.72 ± 1.17	1.17
	Reference method [13]	100.53 ± 1.11	1.10
	Single factor ANOVA: F = 0.59, F (critical) = 3.89		
MLX	**Method I**	99.52 ± 0.45	0.45
	Method II	100.36 ± 1.55	1.54
	Reference method [13]	100.05 ± 1.53	1.53
	Single factor ANOVA: F = 0.55, F (critical) = 3.89		
Standard addition method			
DCN	**Method I**	100.21 ± 0.82	0.82
	Method II	100.05 ± 1.04	1.04
MLX	**Method I**	99.30 ± 0.88	0.89
	Method II	100.34 ± 1.27	1.27

[a] Mean ± standard deviation for five determinations.
[b] % Relative standard deviation.

metrics typically give a good revealing appraisal of the greenness of diverse analytical techniques. Therefore, we use the two novel greenness metrics GAPI and AGREE to decide the greenness of the suggested methods together with other published ones. The GAPI [18] is a comprehensive GAC tool that gives a detailed assessment of different procedures regarding 15 characters of the methods, including all features between the sample collection and the final stage of measurement and volume of waste. These features in the GAPI pictogram are represented as colored zones, either green, yellow, or red color, showing little, medium, and great detrimental environmental effects. Using the GAPI metric, the recently reported HPTLC method[15] had the greatest green sectors and was regarded as the most green procedure (6 green, 7 yellow, and 2 red sectors), then came our spectrophotometric methods together with the other broad spectrum HPTLC one[14] (4 green, 8 yellow and 3 red sectors) and finally the two published HPLC methods[13,14] were found to be the least green procedures (3 green, 8 yellow and 4 red sectors) (**Table 5**). The newer AGREE metric [17] offers a good inclusive appraisal of the environmentally friendliness character of different methods regarding the twelve GAC principles. Using a downloadable calculator, this smart metric can automatically give the final score by simply entering the 12 features in the software. This final score is located in the center of a circle rounded by the 12 individually colored sectors. The closer the final score to unity, the greener the method is. After inputting the 12 variables of each method in the AGREE calculator, the published HPTLC method [15] again acquired the highest score (0.79), giving evidence of its excellent benign character compared with other methods. The suggested spectrophotometric methods and the other reported HPTLC method[14] acquired the second rank (score = 0.68). After that, the two reported HPLC methods [13, 14] had the lowest AGREE scores of 0.63 and 0.55 revealing their huge harmful impact on the environment **(Table 5)**. According to energy consumption issue, the developed spectrophotometric methods are favorable as they consume less than 0.1 kWh/ sample compared to the reported chromatographic methods [13-15] which all consume energy ≥ 1.5 kWh/ sample. Furthermore, the spectrophotometer is available in each quality control lab so, our methods integrate simplicity with availability and minimal energy consumption.

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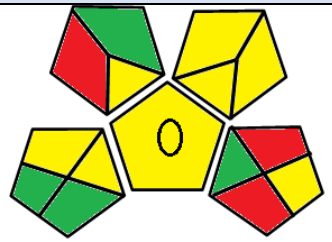
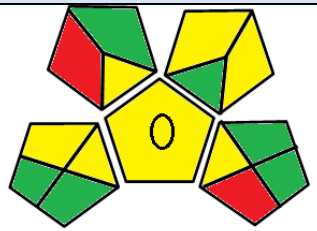
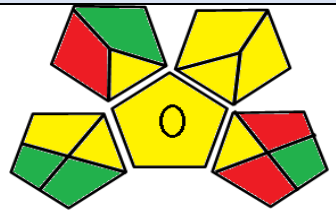
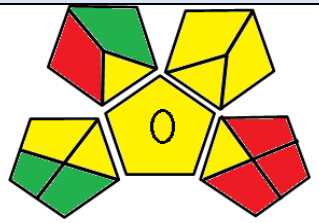
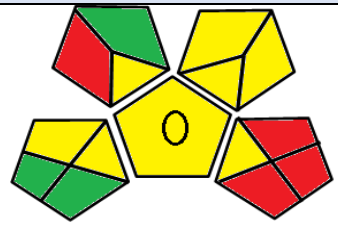
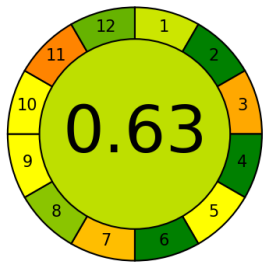
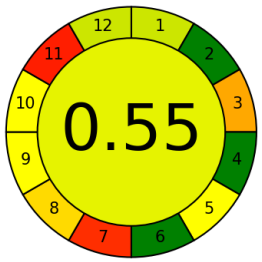
Table 5: Greenness assessment and comparison of the developed method and reported methods using GAPI and AGREE metrics.

parameter	Developed methods	Reported TLC method [15]	Reported TLC method [14]	Reported HPLC method [14]	Reported HPLC method [13]
Mobile phase	Acetonitrile and methanol as solvents	Ethyl acetate: ethanol: water (9.5: 1:0.5)	Chloroform: methanol: acetic acid (92: 8: 0.25)	0.05 M phosphate buffer and acetonitrile (42: 58)	gradient elution of 0.02 M sodium acetate pH 4.5 buffer as mobile phase-A and Acetonitrile and water (9:1) as mobile phase–B
Waste volume	2 mL	0.75 mL	0.75 mL	11 mL	45 mL
GAPI pictogram					
	4 green, 8 yellow, 3 red	6 green, 7 yellow, 2 red	4 green, 8 yellow, 3 red	3 green, 8 yellow, 4 red	3 green, 8 yellow, 4 red
AGREE pictogram					

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3.6. Sustainability evaluation of the developed methods versus published ones

Sustainability is a multifaceted term collecting a thorough overview of an analytical methodology. A sustainable method is a green and benign procedure as well as an economical, readily valid, and analytically efficient method. To evaluate analytical methods' Sustainability, the White Analytical Chemistry" (WAC) [20] concept was introduced with its 12 integrative principles alternatives to the 12 GAC principles. The RGB system evaluates the method whiteness depending on an inclusive appraisal consisting of 4 red (R), 4 green (G), and 4 blue (B) characters associated with the method's analytical effectiveness, its impact on the environment, and its effectiveness in practice and saving-money, respectively thus WAC represents a more pragmatic tool. This RGB 12 is demonstrated as an Excel spreadsheet that facilitates data input and automatically generates simple tables containing the assessment score of each one of the WAC principles along with the net final result illustrating the arithmetic mean of R (%), G (%) and B (%), reflecting the procedure's sustainability. By implementing the RGB assessment of the developed methods and reported procedures, reported HPTLC method [15] acquired the greatest overall whiteness score (95%) followed by adopted spectrophotometric methods (score = 93.5%) and then the broad spectrum HPTLC and HPLC-DAD methods [14] having final scores of 92.8% and 88.5%, respectively. Lastly, the other reported HPLC method [13] obtained the least whiteness score (score = 80.1%) **(Table 6).** It is worth mentioning that the suggested methods have a bit higher blue score than the newly reported HPTLC one [15] which reflects cost and time efficiency, operational simplicity, and easy applicability of the developed methods. Hence the choice between the adopted spectrophotometric methods and the most sustainable HPTLC method [15], possessing comparable whiteness results, depends on the specific requirements of the analysis and the instrument availability.

Table 6: Whiteness assessment and comparison of the developed method and reported methods using the RGB 12 algorithm.

Method name	R (%)	G (%)	B (%)	Whiteness (%)
Developed methods	92.5	92.5	95.4	93.5
Reported TLC method[15]	95.0	95.0	95.0	95.0
Reported TLC method[14]	95.0	88.3	95.0	92.8
Reported HPLC method[14]	95.0	86.7	83.8	88.5
Reported HPLC method[13]	90.0	71.7	78.8	80.1

4. Conclusions

Two sustainable, reliable, simple, and low-cost UV-spectrophotometric procedures, based on simple spectral data handling, were presented for simultaneous quantification of DCN and MLX in their pure forms, synthetic mixtures with different ratios, and pharmaceutical preparation. The suggested methods are accurate, precise, and validated in accordance with ICH guidelines. Reviewing the literature, no spectrophotometric report is available dealing with the concomitant determination of DCN and MLX till now. Both methods can simultaneously analyze the two drugs under investigation where the issue of overlapped spectra was completely resolved without the need for prior separation. The proposed dual wavelength method has the advantages of being simpler and more selective for concurrent estimation of the cited drugs through the direct use of their zero-order spectra, while in the zero-crossing derivative method, we should first extract the derivative spectra of the drugs to solve their overlapping. Moreover, the proposed

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spectrophotometric methods are environmentally friendly, rapid, and simple as it does not involve the high cost and sophisticated methodology associated with different chromatographic methods and these developed methods only depend on the use of a simple spectrophotometer that is readily found in all quality control units.

Assessment of the established methods' greenness and comparison with previously published chromatographic ones was done using two new GAC tools along with whiteness evaluation according to the newly published RGB 12 model. It was proved that the adopted spectrophotometric techniques came in the second rank after the recently reported HPTLC method [15] using GAPI, AGREE, and RGB models. Nevertheless, the privilege of the adopted spectrophotometric procedures resides in their simplicity, cost-effectiveness, and widespread availability in each quality control unit together with minimal negative impacts on the environment. Finally, the proposed methods could be regarded as the most convenient to implement for the determination of this binary mixture in quality control units.

Conflicts of Interest

The authors declare that they have no conflict of interest.

Availability of data and materials

Data and materials are presented in detail in the manuscript. For further information, please contact the authors.

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Authors' contributions

Ass. Prof. Dina S. El-Kafrawy: Conceptualization, Methodology, Writing-review and editing, Supervision.

Dr. Amira H. Abo-Gharam: Methodology, Data analysis – writing – original draft

Prof. Magdy M. Abdel-Khalek: Conceptualization, Data curation, Validation, Supervision.

Prof. Tarek S. Belal: Conceptualization – Writing – review and editing, Formal analysis, Supervision.

Highlights

- Two spectrophotometric methods named the dual wavelength and zero-crossing derivative spectrophotometry techniques were investigated for the concurrent estimation of DCN and MLX in their combined formulation.
- The developed methods were validated in accordance with ICH guidelines and can be easily applied for the determination of this binary mixture in quality control units.
- The suggested methods possess greenness and whiteness properties according to GAPI, AGREE, and RGB approaches.

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