Research article

Green analytical methods for simultaneous determination of compounds having relatively disparate absorbance; application to antibiotic formulation of azithromycin and levofloxacin

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A B S T R A C T

Green validated spectrophotometric methods are developed for simultaneous determination of Azithromycin (AZI) and Levofloxacin (LEVO) antibiotic mixture. Determination of AZI presents a real analytical challenge as its structure lacks any chromophore, and hence it cannot be determined by direct spectrophotometry. However, the reaction of AZI with perchloric acid produces a green product that can be accurately determined spectrophotometrically. Thus, the work presented demonstrates simple green and sensitive methods for the simultaneous determination of AZI and LEVO mixture. Method I depends on direct measurement of absorbance of azithromycin and levofloxacin in perchloric acid methanolic solution at 482 nm and 224 nm, respectively. While, Method II depends on measuring the first derivative spectrophotometric peak-to-peak amplitudes of AZI and LEVO in perchloric acid methanolic solution at 475–490 nm and 280–253 nm, respectively. Regression analysis shows good linearity for AZI and LEVO over the concentration ranges of 5–50 and 2.5–20 µg/mL for method I and 5–50 and 5–40 µg/mL for method II for AZI and LEVO, respectively. The proposed methods were validated in compliance with ICH guidelines. The suggested procedures are successfully applied for the assay of AZI and LEVO mixture in bulk powder and laboratory-prepared tablets. Greenness profile of the proposed methods were compared with other published methods through applying the Eco-scale protocol. Assessment results demonstrated that the proposed methods are greener than other reported methods. Moreover, upon comparison with other methods, the proposed methods showed better or comparable sensitivity in addition to being selective and rapid with no requirement for laborious extraction techniques. These advantages encourage the application of the proposed methods in routine analysis of AZI and LEVO in quality control laboratories as green and simple analytical tool.

1. Introduction

Azithromycin (AZI) (Figure 1), being a member of the macrolides, is structurally related to erythromycin but it is more stable chemically and tolerated than erythromycin [1]. AZI has a broader antimicrobial effect than erythromycin against Haemophilus influenza, Mycobacterium avium complex, Chlamydia trachomatis and nontuberculous mycobacteria. AZI is primarily used for the treatment of patients with mild to moderate respiratory, enteric and genitourinary infections and may be used instead of other macrolides for some sexually transmitted and enteric infections.

Levofloxacin (LEVO) (Figure 1) is a third-generation fluoroquinolone which is a broad-spectrum antibiotic [2]. Levofloxacin has a broader antimicrobial spectrum against Gram-negative, Gram-positive, anaerobes and atypical pathogens than other older generation quinolones such as ciprofloxacin and norfloxacin [3]. LEVO is widely used in the treatment of mild-to-moderate respiratory and urinary tract infections due to sensitive organisms including acute bacterial exacerbations of chronic bronchitis, acute bacterial sinusitis (ABS), nosocomial or community-acquired pneumonia (CAP), urinary tract infections (UTIs), chronic bacterial prostatitis, acute pyelonephritis, skin and skin structure infections.

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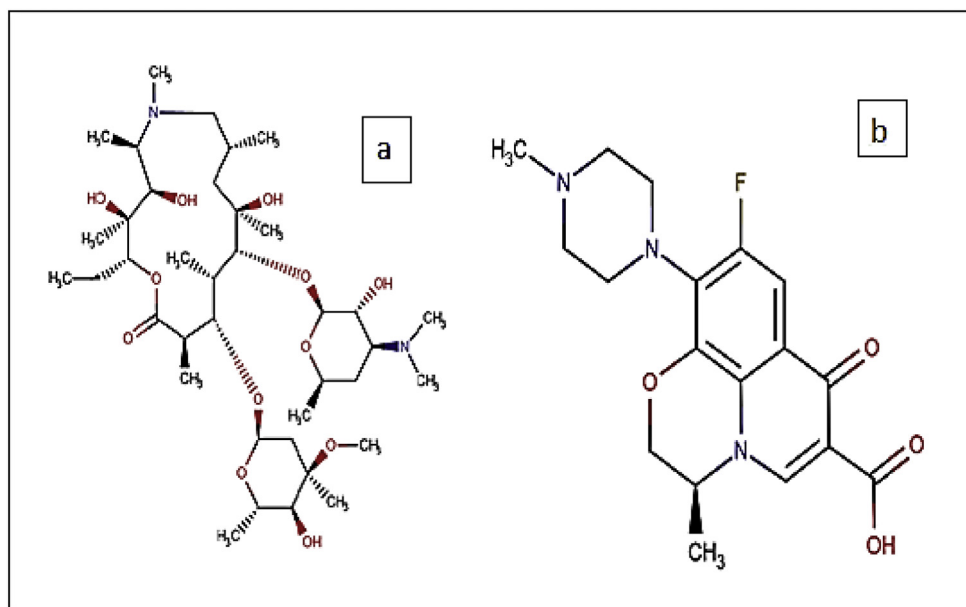   E-mail address: elyazbi@ualberta.ca (A.F. El-Yazbi).

Figure 1. Chemical structure of azithromycin (a) and levofloxacin (b).

A combination of AZI and LEVO has proved to be efficient in the treatment of rheumatoid arthritis. On literature survey, AZI and LEVO have been determined in bulk material and in pharmaceuticals by few different methods, such as spectrophotometry [4, 5, 6] and HPLC [7, 8, 9, 10]. The reason of having such few numbers of publications for the determination of such mixture may be attributed to the lack of any chromophore in the chemical structure of AZI which represents a real analytical challenge. This hinders its determination by direct spectrophotometric methods or by any chromatographic method with direct UV detection.

The aim of the present study is to develop validated spectrophotometric methods for simultaneous determination of AZI and LEVO in pharmaceutical dosage form using green analytical procedures. The proposed methods are performed for the selective spectrophotometric analysis of AZI and LEVO simultaneously based on the oxidative interaction of the selected drugs with perchloric acid to produce a green oxidation product with AZI without any interference from LEVO. **Method I** depends on direct measurement of absorbance of AZI and LEVO in perchloric acid methanolic solution. **Method II** depends on measuring the first derivative spectrophotometric peak-to-peak amplitudes of AZI and LEVO in the same perchloric acid methanolic solution. The developed methods are simple and highly reproducible for the simultaneous determination of the studied drugs with high selectivity and sensitivity. Moreover, implementation of the analytical eco-scale protocol was accomplished in order to examine the greenness of the proposed methods and was compared to the other reported methods that did not contemplate any green analytical practices, specifically, in terms of hazardous chemicals, waste production and energy consumption [11, 12]. Upon comparison of the proposed methods with other reported methods [4, 5, 6, 7, 8, 9, 10], the former proved better comparable sensitivities for the determination of the studied drugs with the advantage of being simpler and greener. Therefore, they can be readily applied for quality control purposes as an eco-friendly, simple and efficient analytical tool.

2. Experimental

2.1. Instrumentation

Measurements were performed using a thermospectronic UV-VIS spectrometer (Helios alpha, Unicam, England). All measurements were performed in 1-cm quartz cells. The instrument was connected to vision32 software. The absorbance data were manipulated using Excel™ software.

2.2. Materials and reagents

Reference azithromycin dihydrate (99.05%) and levofloxacin hemihydrate (purity 98.92%) were purchased from Pharco pharmaceutical company and Pharo Pharma Company, respectively. Perchloric acid 70% (a product of Qualikems Fine Chem Pvt.Ltd (India)) and methanol (a product of Sigma-Aldrich Chemie GmbH (Germany)) were analar grade. Laboratory-prepared tablets were prepared by mixing AZI and LEVO well in ratio of 1:1 with excipients (talc powder, dibasic calcium phosphate and aerosol) and compressed as tablets.

2.3. General procedure and construction of calibration graphs

2.3.1. Preparation of standard solutions and construction of calibration graphs

The standard stock solutions 1 mg/mL of AZI and LEVO were prepared separately in methanol. A working stock of 100 μg/mL was prepared in methanol.

2.3.1.1. Method I: direct absorbance measurement. Into two separate sets of 10-mL volumetric flasks, measured volumes were accurately added followed by 3 mL perchloric acid. Dilution was made to volume with methanol after 10 min to obtain the concentration ranges from 5 to 50 μg/mL for AZI and 2.5–20 μg/mL for LEVO. The absorbance was measured at 482 nm for AZI and 224 nm for LEVO against blank. The
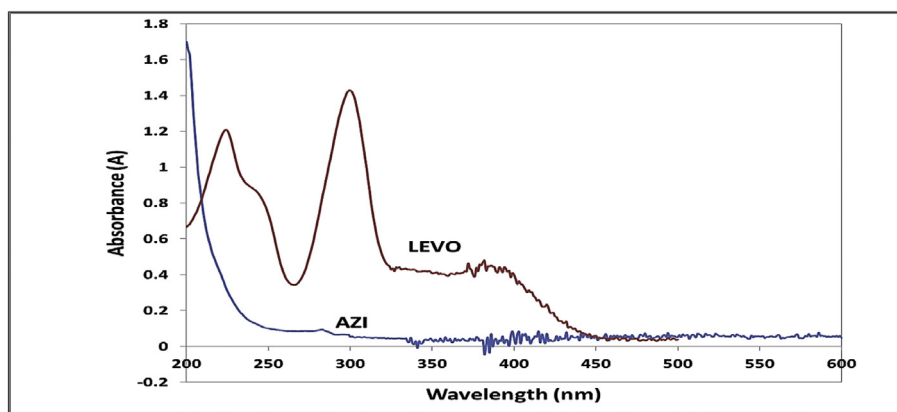
Figure 2. Zero order absorption spectrum of 20 μg/mL AZI and 20 μg/mL LEVO in methanol.
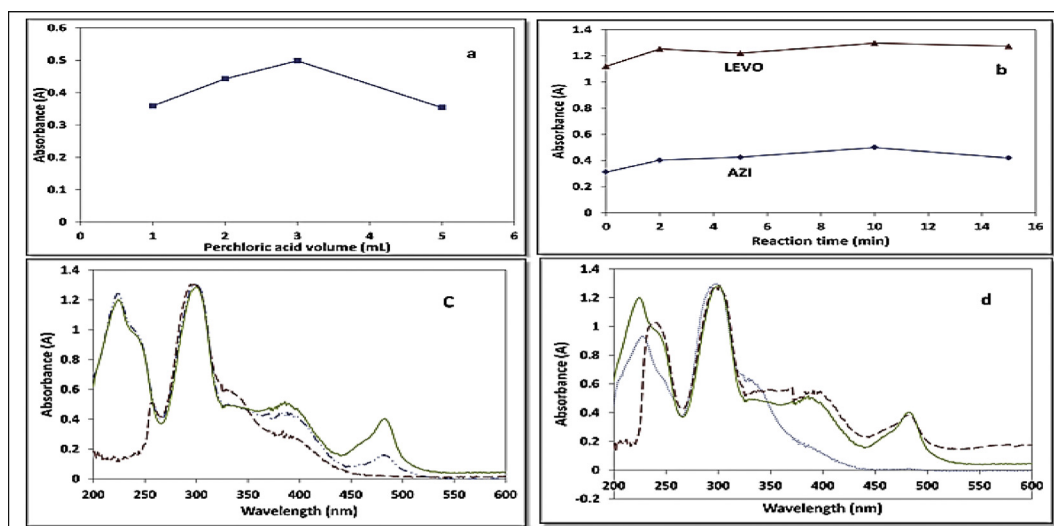

Figure 3. (a) Studying the effect of perchloric acid volume, (b) the effect of reaction time, (c) the effect of different solvents, methanol (———), water (- .. - .. - .. -) and DMF (- - - -), (d) the effect of different oxidizing acids, perchloric acid (———), sulfuric acid (- - - -) and phosphoric acid (……..) on the oxidative reaction product of 20 μg/mL of AZI and LEVO.

absorbance values were plotted against concentration to obtain calibration curves.

2.3.1.2. Method II: first derivative spectrophotometric method.
The absorption spectra of the prepared final working standard solutions were scanned from 200 to 600 nm. Data were manipulated to record the first derivative (D_1) spectrum of each solution. D_1 amplitudes were measured from peak to peak at (475–490 nm) and (280–253 nm) for AZI and LEVO, respectively, with linearity range 5–50 μg/mL for AZI and 5–40 μg/mL for LEVO. To obtain the calibration graphs, absolute derivative values at the selected wavelengths were plotted against the corresponding concentration of each drug. After that, the corresponding regression equations for each drug were derived.

2.3.2. Determination of the synthetic mixtures
Laboratory-prepared mixture of AZI and LEVO were prepared using aliquots of AZI and LEVO stock solutions equivalent to 5–40 μg/mL and 5–20 μg/mL, respectively, to check the repeatability and reproducibility of the proposed methods.

2.3.3. Analysis of laboratory-prepared tablets
Two laboratory-prepared tablets (each tablet labelled to contain 250 mg LEVO and 250 mg AZI) were crushed and mixing well. Then 1000 μg/mL sample solution was prepared from the laboratory-prepared tablets in 100-mL volumetric flask where thirty milliliters of methanol were added and the flask was sonicated for 30 min. Then dilution was made to volume with methanol. The solution was filtered twice through a 0.45-μm membrane filter to get a clear solution. After filtration, a working solution (100 μg/mL) was prepared in methanol. Three different determinations were selected within the linearity range and measured following the procedure above.

3. Results and discussion

The determination of AZI represents a real analytical challenge as its structure lacks any chromophore, and hence it cannot be determined by direct spectrophotometric methods as shown in its absorption spectrum (Figure 2).

In literature, perchloric and sulfuric acids have been added as reagents on certain macrolides such as erythromycin, spiramycin,
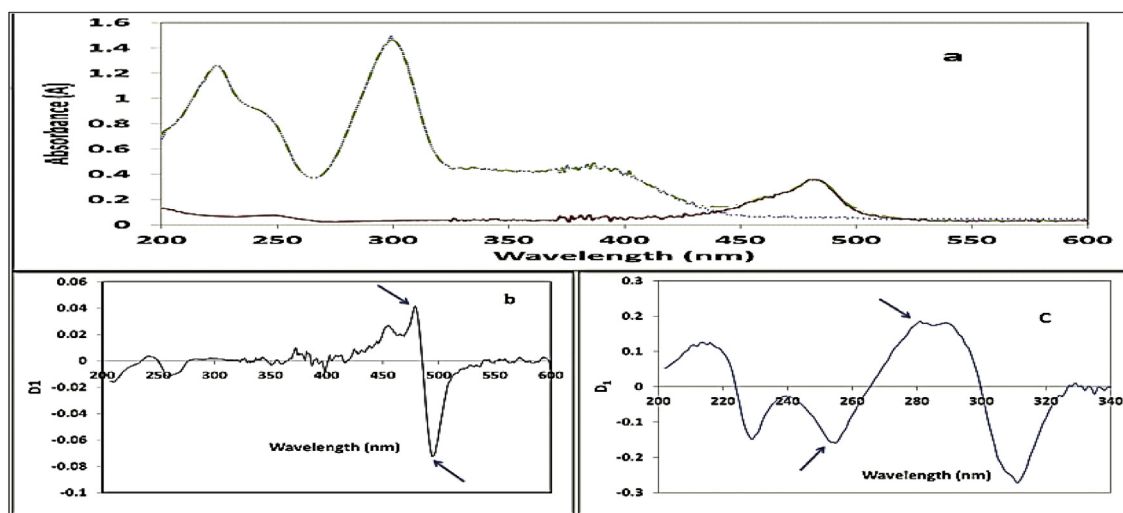
Figure 4. (a) Zero order absorption spectra of 20 μg/mL AZI (——), 20 μg/mL LEVO (……..) and their mixture (- .. - .. - .. -) in methanol after interaction with perchloric acid, (b) corresponding first derivative spectrum of 20 μg/mL AZI, (c) corresponding first derivative spectrum of 20 μg/mL LEVO. First derivative spectra of 20 μg/mL AZI curve is smoothed using Microsoft excel™ software. Arrows indicate the selected points for analysis.

troleandomycin and oleandomycin for some spectrofluorimetric and spectrophotometric methods. Perchloric and sulfuric acids help to create chromophores to determine some macrolides [13, 14, 15, 16]. They promote breaking of large particles into smaller ones with greater quantum yield [16]. Perchloric acid (E0 = 1.75 V) is stronger than sulfuric acid (E0 = 1.44 V) and has a higher oxidative power.

The proposed spectrophotometric methods rely on the reaction of perchloric acid with AZI to produce a green oxidation product with maximum wavelength at 482 nm without any interference from LEVO as shown in Figure 3. The produced oxidation product has chromophores that are easily determined by spectrophotometry. This allows the accurate determination of AZI.

3.1. Optimization of reaction conditions

Different factors were found to affect the intensity of the green product. These factors must be optimized to ensure complete oxidation. The optimization was performed using 20 μg/mL working standard solutions of LEVO and AZI.

3.1.1. Perchloric acid volume

Different volumes of perchloric acid were studied using the previously mentioned procedure. Optimum color intensity and reproducible maximum wavelength were produced with 3 mL perchloric acid (Figure 3a). This indicates complete oxidation.

3.1.2. Effect of reaction time

Different time intervals were studied (0–15 min). The maximum color intensity was obtained after 10 min as shown in Figure 3b.

3.1.3. Solvent selection

Different solvents such as methanol, water and DMF have been studied according to the procedure mentioned above. Optimum color intensity and higher sensitivity were obtained with methanol (Figure 3c).

3.1.4. Type of the oxidizing acid

Different oxidizing acids were studied such as perchloric acid, sulfuric acid and phosphoric acid (Figure 3d). The results showed that perchloric acid and sulfuric acid succeeded in performing oxidation of AZI while phosphoric acid failed to. In this work, perchloric acid is selected over sulfuric acid due to its higher oxidative power that provided better stability of the produced oxidation product.

3.2. Simultaneous determination of AZI and LEVO

3.2.1. Method I: absorbance measurements

This method depends on measuring the absorbance values directly for LEVO at λ_{max} of 224 nm and for AZI oxidation product following its reaction with perchloric acid at λ_{max} of 482 nm (Figure 4a).

3.2.2. Method II: first derivative spectrophotometric method

For the first derivative spectrophotometric method (D_1 method), the ratio of D_1 values between (475–490 nm) and (280–253 nm) as shown in Figure 4b, c for AZI and LEVO, respectively, were calculated in order to detect the presence of any interferences. The results for different concentrations of AZI and LEVO standard solutions and laboratory-prepared tablets are indicated in Table 1. The obtained results show RSD% values less than 2% indicating that this method is specific for the studied drugs and can be used to test their identity and purity.

Table 1. Ratio of D_1-spectrophotometric peak amplitudes for different concentrations of AZI and LEVO in bulk powder and in laboratory-prepared tablets.

Concentration (μg/mL)		Bulk powder		Laboratory-prepared tablets	
AZI	LEVO	AZI $D_{1\ 475nm}/D_{1\ 490nm}$	LEVO $D_{1\ 280nm}/D_{1\ 253nm}$	AZI $D_{1\ 475nm}/D_{1\ 490nm}$	LEVO $D_{1\ 280nm}/D_{1\ 253nm}$
10	10	0.60	1.14	0.61	1.13
20	20	0.58	1.14	0.60	1.13
30	30	0.60	1.12	0.60	1.11
Mean		0.59	1.13	0.60	1.12
SD		0.01	0.01	0.01	0.01
RSD%		1.69	0.88	1.67	0.89

Table 2. Regression and statistical parameter for the determination of AZI and LEVO using the proposed methods. S_a is standard deviation of intercept, S_b is standard deviation of slope and $S_{y/x}$ is standard deviation of residuals.

Parameters	Method I (Direct)		Method II (D_1)	
	AZI	LEVO	AZI	LEVO
Linearity range (µg/mL)	5.00–50.00	2.50–20.00	5.00–50.00	5.00–40.00
LOQ (µg/mL)	4.69	2.43	4.47	4.56
LOD (µg/mL)	1.55	0.80	1.47	1.50
Intercept (a)	1.72×10^{-2}	9.35×10^{-3}	5.68×10^{-3}	-2.59×10^{-3}
Slope (b)	1.71×10^{-2}	5.95×10^{-2}	5.72×10^{-3}	1.67×10^{-2}
Correlation coefficient (r)	0.9996	0.9991	0.9995	0.9992
S_a	6.30×10^{-3}	1.24×10^{-2}	1.99×10^{-3}	6.55×10^{-3}
S_b	2.07×10^{-4}	1.01×10^{-3}	6.55×10^{-5}	2.66×10^{-4}
S_b^2	4.28×10^{-8}	1.02×10^{-6}	4.29×10^{-9}	7.08×10^{-8}
$S_{y/x}$	8.02×10^{-3}	1.45×10^{-2}	2.56×10^{-3}	7.62×10^{-3}
F	6828.17	3470.70	7624.17	3951.43
Significance F	3.91×10^{-6}	1.08×10^{-5}	1.03×10^{-7}	8.87×10^{-6}

3.3. Validation of the proposed methods

Validation of the proposed methods was carried out in compliance with the International Conference on Harmonization (ICH) guidelines [17].

3.3.1. Linearity and concentration ranges

Calibration graphs are obtained by plotting absorbance and first derivative responses at the selected wavelengths versus concentrations for both AZI and LEVO. Table 2 demonstrates the results for regression and statistical parameters. The linear equation data show high correlation coefficient values (R > 0.999) with small intercepts. The variances around the slopes (S_b^2) with low values show the small degree of scattering of experimental points around the regression line. The high F-values indicate a decrease in the mean squares due to residual and an increase in the mean squares due to regression. The small values of the mean squares due to residual indicate less scattering of the experimental data points around the line of regression. In addition, the greater the values of the mean square due to regression, the more the steepness of the line of regression. So, the high F-values (low significance F) of regression lines are better than regression lines with small values.

3.3.2. Detection and quantification limits

The detection limit (DL) and quantitation limit (QL) were calculated according to the ICH guidelines equations:

$$DL = 3.3 \, \sigma/S$$

$$QL = 10 \, \sigma/S$$

where, σ is the standard deviation of the residuals and S is the slope of the calibration curve. For the proposed methods, low values of DL and QL were obtained for each drug indicating the sensitivity of the proposed methods as presented in Table 2.

3.3.3. Accuracy

Three synthetic mixtures of the selected drugs were prepared with different ratios according to the linearity ranges. Analysis of each synthetic mixture was performed three times (n = 3). Accuracy was studied by the analysis of each drug in the presence of the other drug in synthetic mixtures and was calculated from the obtained results as the average recoveries of the analyte assayed. Good recoveries and low percentage error indicated the high accuracy of the proposed methods (Table 3).

3.3.4. Intra-day and inter-day precision

Three concentrations of both AZI and LEVO were analyzed three times each on the same day (intra-day) and the precision is calculated as the relative standard deviations. A similar procedure was performed to estimate the inter-day precision but on three consecutive days (Table 3).

Table 3. Intra-day and inter-day precision and accuracy for the determination of AZI and LEVO mixtures using the proposed spectrophotometric methods.

Concentration (µg/mL)		Method	Recovery % ± SD[a]		RSD%[b]		$E_r\%$[c]	
AZI	LEVO		AZI	LEVO	AZI	LEVO	AZI	LEVO
			(a) Accuracy and intra-day precision					
10	20	Direct	101.05 ± 0.59	100.95 ± 0.26	0.58	0.25	1.05	0.95
		D_1	100.53 ± 1.75	100.32 ± 0.69	1.75	0.69	0.53	0.32
40	10	Direct	100.75 ± 0.81	100.79 ± 0.12	0.79	0.12	0.75	0.97
		D_1	99.99 ± 0.91	100.32 ± 0.69	0.91	0.69	-0.01	0.32
20	20	Direct	99.69 ± 0.44	100.76 ± 0.91	0.44	0.91	-0.31	0.76
		D_1	100.85 ± 0.51	101.58 ± 0.91	0.51	0.90	0.85	1.58
			(b) Accuracy and inter-day precision					
10	20	Direct	101.44 ± 0.89	100.89 ± 0.36	0.88	0.35	1.44	0.87
		D_1	99.36 ± 1.01	99.92 ± 0.53	1.02	0.53	-0.64	-0.08
40	10	Direct	100.02 ± 0.22	100.83 ± 0.33	0.22	0.33	0.02	0.83
		D_1	100.57 ± 1.16	100.25 ± 0.64	1.15	0.64	0.57	0.25
20	20	Direct	101.53 ± 1.12	98.70 ± 0.69	1.09	0.69	1.53	-1.29
		D_1	101.14 ± 0.88	101.27 ± 1.13	0.87	1.12	1.14	1.27

(a) is standard deviation, (b) is percentage relative standard deviation and (c) is percentage error.

Table 4. Results for the analysis of the synthetically prepared mixtures containing AZI and LEVO using the proposed spectrophotometric methods.

Concentration (μg/mL)		Recovery% ± RSD%	
AZI	LEVO	AZI	LEVO
5	10	98.01 ± 1.19	100.39 ± 0.45
10	20	101.05 ± 0.58	100.95 ± 0.25
10	5	101.83 ± 0.33	99.04 ± 0.38
20	20	100.85 ± 0.50	101.58 ± 0.90
20	10	100.72 ± 1.37	100.22 ± 0.62
40	20	100.75 ± 0.79	100.79 ± 0.12
40	10	101.38 ± 1.67	98.74 ± 0.84
Mean recovery (%)		100.72 ± 0.92	100.24 ± 0.51

Table 5. Evaluation of robustness of the proposed methods for the determination of AZI and LEVO mixtures.

Parameters	Method	Recovery % ± RSD %	
		AZI	LEVO
Maximum wavelength λ max ±2 nm	Direct	98.64 ± 0.48	99.13 ± 0.30
Reaction time 10 ± 1 min	Direct	99.13 ± 0.24	99.08 ± 0.46
	D_1	100.42 ± 0.54	101.02 ± 0.26
Perchloric acid volume 3 ± 0.1 mL	Direct	99.19 ± 0.25	98.85 ± 1.14
	D_1	100.14 ± 0.94	99.64 ± 0.68

The obtained results show that RSD% is less than 2%. This indicates the high degree of precision of the proposed methods.

3.3.5. Selectivity

The methods selectivity was studied by the analysis of laboratory/synthetically prepared mixtures which contain different ratios of the studied drugs. The ratios were selected to be below and above their normal concentration in the pharmaceutical formulation together with the inactive excipients. The results showed acceptable recoveries and good values of precision for different ratios of AZI and LEVO. The precision and accuracy of the methods were indicated in Table 4. These ensure that the analytical methods are powerful to determine and resolve the investigated drugs in different proportions.

3.3.6. Stability of solutions

The stability of sample solutions was carried out for a mixture of 100 μg/mL LEVO and AZI at 4 °C for one week. The analysis of these solutions indicated the stability of both drugs under these conditions without spectrophotometric changes, which is no significant changes were observed in maximum wavelengths or absorbance values for the selected drugs. The values of %RSD did not exceed 2%. Also, stock solutions were found to be stable for at least two weeks in refrigerator. The calculated concentrations of recently prepared and aged solutions for 2 weeks were analyzed using the proposed method. The difference found to be less than 2.0%.

3.3.7. Robustness

Studying robustness of the proposed methods was performed by small deliberate variations in the parameters of the methods such as maximum wavelength, reaction time and perchloric acid volume. Analysis of triplicate injections with only a single change was applied. The method proved to be robust, as the studied changes did not significantly affect the absorbance values of the selected drugs. This was verified by RSD% values that did not exceed 2%. Mean recovery % and RSD% indicate high degree of reliability of proposed methods (Table 5).

3.4. Analysis of the synthetic mixtures

Different ratios of AZI and LEVO were selected within the linearity range to prepare the synthetic mixtures. The results of analysis showed no interference between the absorption spectra of the drugs. This proved good accuracy and precision of the proposed methods represented in E_r % and RSD% values.

3.5. Analysis of laboratory-prepared tablets

The proposed methods were applied for the assay of the combination of AZI and LEVO in laboratory-prepared tablets. Each drug was analyzed at its specific λ_{max}. Results of analysis provided satisfactory recovery% and RSD% values (Table 6). These results proved that the proposed methods are applicable for analysis of both active ingredients in

Table 6. Application of the proposed methods for the simultaneous determination of AZI and LEVO with ratio 1:1 in laboratory-prepared tablets for five determinations.

Pharmaceutical preparation	Recovery% ± % RSD					
	Method I (Direct)		Method II (D_1)		Reported HPLC Method [7]	
	AZI	LEVO	AZI	LEVO	AZI	LEVO
Laboratory-prepared tablets	100.49 ± 1.45	100.44 ± 0.94	100.58 ± 0.92	100.12 ± 0.89	99.77 ± 0.78	99.80 ± 0.59
t [a]	0.97 (2.45)	1.28 (2.36)	1.50 (2.31)	0.67 (2.36)		
F [a]	3.51 (9.61)	2.61 (9.61)	1.43 (9.61)	2.32 (9.61)		

[a] Figures between parentheses represents the corresponding tabulated values of t and F at P = 0.05.

Table 7. The penalty points for the determination of AZI and LEVO for the proposed methods and the reported methods.

Reagents/instruments	Penalty points		
	Proposed Methods	Reported method [6]	Reported method [7]
Methanol	6	-	18
Acetonitrile	-	4	-
Chloroform	-	2	-
Benzene	-	6	-
Ortho-phosphoric acid	-	-	6
Perchloric acid	4	-	-
DDQ	-	2	-
Chloranilic acid (p-CA)	-	1	-
Crystallization and filtration	-	1	-
UV-VIS spectrophotometry	0	0	-
HPLC	-	-	1
Occupational hazard	0	0	0
Waste >10mL	5	5	5
Total penalty points	15	21	30
Analytical Eco-Scale total score	85	79	70

Table 8. Collective comparison between the developed and the reported methods for the simultaneous determination of AZI and LEVO mixtures.

Analytical methods		λ(nm)		Linearity range (μg/mL)		Correlation coefficient		LOD		Comments
		AZI	LEVO	AZI	LEVO	AZI	LEVO	AZI	LEVO	
Proposed method I		482	224	5.0–50.0	2.5–20.0	0.9996	0.9991	1.55	0.80	*No need to adjust pH
Proposed method II		475–490	280–253	5.0–50.0	5.0–40.0	0.9995	0.9992	1.47	1.50	*No need of pre-treatment or extraction *Non-destructive
Reported method [4]		536	301	4.0–20.0		0.973	0.9983	1.07	0.84	*Lengthy
Reported method [6]	Method A	390–650 (AUC)		12.0–225	10.0–200	0.999	0.998	1.4	1.0	*Buffer pH adjustment (apt to personal and/or instrumental variations which may affect the ruggedness of the methods)
	Method B	400–700 (AUC)		40.0–500	30.0–300			5.0	3.0	
Reported method [5]	Method I	245	290	20.0–100		0.9971	0.9975	6.0	8.52	*Mandatory filtration of samples and mobile phase
	Method II	245	290					4.08	9.50	*Expensive organic solvents
Reported method [7]		269		50–150		0.99	0.99	34.5	43.6	
Reported method [8]		285		50.0–150.0		0.9991	0.999	20.50	18.25	
Reported method [9]		285		50.0–150		0.99	0.99	6.13	5.47	
Reported method [10]		262		20.0–100	2.0–10.0	0.999	0.999	0.01	0.001	

laboratory-prepared tablets with minimum sample preparation and with a satisfactory level of selectivity, accuracy, and precision.

Results obtained were statistically compared with the reported HPLC method [7] using the Student's t-test, and the variance ratio F-test to examine whether the two methods were significantly different or not. The obtained t and F values did not exceed the critical ones (Table 6), which indicated high degree of agreement between the proposed methods and the reported methods.

3.6. Greenness of the methods

Green analytical chemistry is essential in assessing the environmental impact of various analytical procedures. The greenness of the analytical methods was determined according to occupational hazards, amounts of reagents and solvents, waste generation and energy consumption. These factors were involved in calculation of penalty points. The penalty points of hazards are established on the Globally Harmonized System of Classification and Labeling of Chemicals (GHS). Any chemical reagent has one or more pictogram, which is a graphic expression of its hazardous properties. For each reagent, the hazard penalty points are calculated by multiplying number of hazardous pictograms by degree of hazard (multiplication by 1 for 'warning' and multiplication by 2 for 'danger') [18]. The lower the penalty points the higher Eco-Scale score. This indicates that the analytical method is greener and more Eco-friendly.

Eco-Scale score is calculated by subtracting the penalty point values from the basis of 100 points. The green analysis is considered ideal if eco-scale has values of 100, excellent and acceptable if more than 75 and 50, respectively, but inadequate if less than 50 [19].

The comparison between the reported methods and proposed methods according to the analytical Eco-Scale scores is designed (Table 7). The results show that the proposed spectrophotometric method is more eco-friendly than the reported methods. This is due to the higher Eco-Scale score value of the proposed methods. Also, it is obvious that both the proposed and reported spectrophotometric methods score eco-scale values are more than 75. This proves that the spectrophotometric methods generally are excellent green and Eco-friendly methods with minimal requirements, whereas the HPLC reported methods are just acceptable because of the high consumption of the organic solvent and higher energy consumption.

3.7. Comparison with reported methods

As shown in Table 8, the proposed methods offer several advantages over reported spectrophotometric methods [4, 5, 6] and reversed-phase HPLC methods [7, 8, 9, 10]. The present methods are simpler and more economic than HPLC ones which require higher cost for each analysis, large quantity of expensive organic solvents, pH adjustment of used buffers and pre-treatment of samples. Also, the proposed methods

can be beneficial in testing the purity of the drugs and routine analysis of quality control laboratories. Moreover, they provide optimum sensitivity without any necessity for complicated and expensive instrumentation.

4. Conclusion

In this work, validated spectrophotometric methods were developed for simultaneous determination of AZI and LEVO in laboratory-prepared tablets using green analytical procedures. Results show that the proposed methods are selective and rapid with superior sensitivity and greenness over other reported methods. Moreover, they have the advantage of using the spectrophotometer which is readily available in all quality control laboratories. In addition, the proposed methods are selective, sensitive and rapid with no requirement for laborious extraction techniques. These advantages encourage their application in routine analysis of AZI and LEVO in quality control laboratories.

Declarations

Author contribution statement

A. El-Yazbi, E.F. Khamis, R.M. Youssef and M.A. El-Sayed: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

F.M. Aboukhalil: Performed the experiments; Analyzed and interpreted the data.

Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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