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Spectral analysis of overlapped absorption bands of binary mixtures—an application on combination of pseudoephedrine sulphate and loratadine mixture

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Abstract

Background: Simple, specific, accurate, and precise spectrophotometric methods are progressed and validated for concurrent analysis of pseudoephedrine sulphate (PSE) and loratadine (LOR) in their combined dosage form depending on spectral analysis procedures. In this binary mixture, pseudoephedrine (PSE) could be determined by using its resolved spectrum of zero-order absorption at 256.8 nm after subtraction of the spectrum of LOR, and also it could be determined in existence of the spectrum of LOR by different methods including absorption correction method (AC) at 256.8 nm and 280 nm, dual wavelength method (DW) at 254 nm and 273 nm, induced dual-wavelength method (IDW) at 230 nm and 263 nm, and ratio difference method (RD) at 256.8 nm and 270 nm. Loratadine (LOR) in the binary mixture could be determined either by direct analysis at 280 nm without any contribution from the spectrum of PSE or through its recovered spectrum of zero-order absorption via constant multiplication method (CM) using plateau region (277–326 nm). Also, concurrent determination for PSE and LOR in their overlapped binary mixture could be achieved by applying induced amplitude modulation (IAM) method.

Results: Specificity of the proposed spectrophotometric methods was examined by the analysis of prepared mixtures in laboratory and was applied successfully for pharmaceutical dosage form analysis which has the cited drugs without additive contribution. The proposed spectrophotometric methods were also validated as per the guidelines of ICH. Statistical comparison was performed between the obtained results with those from the official methods of the cited drugs, using one-way ANOVA, *F* test, and Student's *t* test, and the results exhibit insignificant difference concerning precision and accuracy.

Conclusions: The previously proposed spectrophotometric methods could be easily used accurately and precisely for simultaneous determination of the studied binary mixture with simple manipulation procedures.

Keywords: Absorbance correction, Constant multiplication, Dual-wavelength, Induced amplitude modulation method, Induced dual-wavelength, Loratadine, Pseudoephedrine sulphate, Ratio difference

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Background

Combinations of pseudoephedrine sulphate (PSE) with loratadine (LOR) are frequently prescribed to treat cold and relieve symptoms of allergic rhinitis due to seasonal changes [1].

Pseudoephedrine (PSE) [(S, S) methylamino-1-phenylpropan-1-ol] as in Fig. 1a acts as a sympathomimetic agent so it could be used as a decongestant drug [1]. Various spectrophotometric methods were established for the determination of pseudoephedrine [2–4]. Several chromatographic methods were applied for the determination of PSE containing HPLC [5–10], HPTLC [11–13], GC [14, 15], and CE [16, 17], and also, many electrochemical methods were reported [18–20].

Antihistamines are used to treat allergic reactions. One of their major side effects is the sedating effect which makes restriction for their use and so second-generation antihistamines commonly prescribed now. Loratadine (LOR) [ethyl 4-(8-chloro-5, 6 -dihydro-11H-benzo 5, 6 cyclohepta [1, 2] pyridin-11-ylidene)-1-piperidinecarboxylate] as in Fig. 1b is a non-sedating, long-acting second-generation H_1 receptor blocker. It is used for treatment of allergy cases including urticaria and rhinitis [1].

Various methods were established for analysis of LOR and its metabolite (desloratadine) in pharmaceutical formulations and in plasma of human containing spectrophotometric methods [2, 21, 22] and chromatographic methods including HPLC [6, 9, 23–25], HPTLC [12, 13, 26], and GC [27, 28]. In addition, several electrochemical and polarographic methods were reported [29–32].

The survey of the literature shows different analytical methods for concurrent analysis of both PSE and LOR. Various chromatographic methods were established for the determination of the two analytes [33–35]. Other methods contain multi-wavelength spectroscopy [35], chemometric treatment of spectrophotometric data [36], first derivative spectroscopy [33].

The aim of this work is to create simple spectrophotometric methods which were able to resolve the spectral overlapping of PSE and LOR. The proposed spectrophotometric methods could be easily used accurately and precisely for simultaneous determination of the studied binary mixture with simple manipulation procedures. The proposed methods could be used in the analysis of their market formula named Clarinase® tablet. A comparative study was made between the obtained results to check the efficiency of the proposed methods in the estimation of the cited drugs.

Theoretical background

Induced amplitude modulation method (IAM)

This method [37] is an extension for amplitude modulation method (AM) and advanced amplitude modulation (AMM) [37].

Ratio spectra of the mixture was obtained by dividing the mixture by the normalized spectrum of the extended drug as a divisor (Y'); this normalized spectrum is obtained by dividing the entire spectrum of Y by its relative concentration to get a new spectrum which acts as the desired analyte's absorptivity (a_Y) against all the wavelengths which had been measured. This method can be clarified by using the following equations:

$$\because \quad [A_1]/[a_Y] = [a_X C_X]/[a_Y] + [C_Y]/[C'_Y] \tag{1}$$

$$\therefore P_{Mix} = a_r C_X + C_Y \tag{2}$$

where P_{Mix} is the recorded amplitude of X and Y mixture and a_{r} is the absorptivity ratio $\{[a_X]/[a_Y]\}$ which is obtained from the division of the normalized spectra of both pure component X and Y, conjointly, while C_Y is the amplitude which is recorded at the extended region which parallel to axis of wavelength

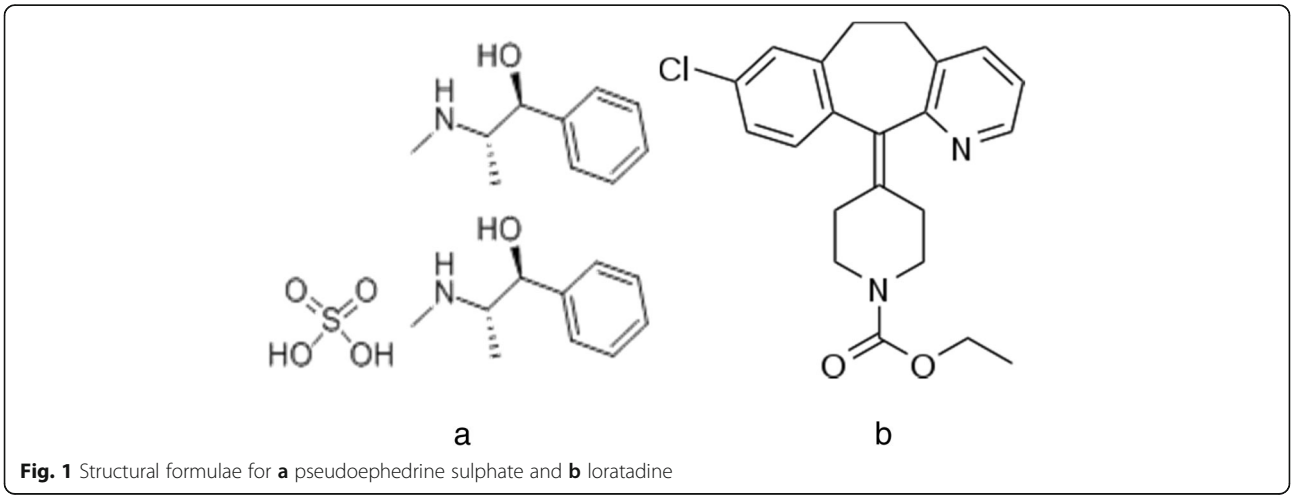

Fig. 1 Structural formulae for **a** pseudoephedrine sulphate and **b** loratadine

which could be modulated directly to the recorded concentration of Y.

Contribution of Y is canceled by subtraction of $[C_Y]/[C_{Y'}]$ to get $[a_X C_X]/[a_Y]$ followed by multiplication by spectrum $[a_Y]/[a_X]$ which is the ratio spectrum of the normalized spectra of Y and X:

$$P_{(X)} = [a_X C_X]/a_Y * [a_Y]/[a_X] \quad (3)$$

$$\therefore P_{(X)} = C_X \quad (4)$$

The concentration of both X and Y could be modified to the actual concentration via substitution in regression equation which represent the amplitude of the ratio spectra of different concentrations of X and Y, separately using normalized Y as a divisor.

$$C_{Recorded} = slope \;\; C_{Actual} \pm intercept \quad (5)$$

The slope here is around unity, and the intercept is approximately equal zero. C_{Recorded} is the amplitude which have been modulated correlative to the concentrations of X or Y.

Experimental

Devices and operating system

The analysis was performed using a Shimadzu UV-1800 spectrophotometer attached to ACER computer. Quartz cells (1.0 cm) were used for recording the absorption spectra of both reference and test solutions at the range of 200–400 nm.

Materials

Authentic materials

PSE and LOR were kindly given by Mina Pharm Company. The purity was detected as 99.56 ± 0.42 and 100.06 ± 0.75 for PSE and LOR, conjointly as stated in the official USP methods [38].

Dosage form

Clarinase® tablets were used with batch number 4JRPG24B02. Every tablet is claimed to have 120.0 mg PSE and 5.0 mg LOR, produced by Schering-Plough Company and obtained from the local market.

Chemicals and solvents

Hydrochloric acid was kindly supplied from El Nasr Chemicals & Pharmaceutical Company, Cairo, Egypt, while distilled water was obtained using Ultra-Pure Water System "Aquatron" (Staffordshire, England) apparatus.

Standard solutions

Standard stock solutions

Preparation of stock solutions of PSE (1500.0 μg/mL) and LOR (100.0 μg/mL) was carried out by dissolving 150.0 mg and 10.0 mg of PSE and LOR, conjointly in 0.1 M HCl in100-mL volumetric flask; then, the volume was adjusted till the mark by using the same solvent and kept at the refrigerator.

Procedure

Spectrophotometric scanning of PSE and LOR

Solutions of PSE (240.0 μg/mL) and LOR (10.0 μg/mL) were prepared using their standard stock solutions (1500.0 μg/mL) for PSE and (100 μg/ mL) for LOR and scanned in a wavelength region 200–400 nm against 0.1 M HCl as blank; then, their spectra of zero-order absorption were obtained.

Construction of calibration graphs

Aliquots equivalent to 1800.0–12,000.0 μg PSE and 50.0–300.0 μg LOR had been transmitted from their stock solutions of PSE (1500.0 μg/mL) and LOR (100.0 μg/mL) to two separate sets of volumetric flasks (10 mL); then using 0.1 M HCl, the volume was adjusted till the mark. Scanning of the spectra of absorption of previously performed standard solutions was conducted in the region from 200–400 nm and kept directly at the computer. The calibration graphs were done using the average of three experiments.

The calculation of the absorption factor was performed by taking the average ratio of absorbance at 256.8 nm and 280 nm ($A_{256.8\text{ nm}}/A_{280\text{ nm}}$) of different concentrations of LOR. In addition, the equality factor of LOR was calculated by taking the average ratio of absorbance at 230 nm and 263 nm ($A_{230\text{ nm}}/A_{263\text{ nm}}$) of different concentrations of LOR.

Calibration graphs of zero-order spectra based on absorbance for SS, DW, IDW, D^0, and CM Construction of calibration graphs of PSE was carried out by plotting the following: the absorbance at 256.8 nm for SS and the absorbance difference between (254 nm, 273 nm) for DW and $[A_{263\text{ nm}} - (\text{eq. factor})_{\text{LOR}}\, A_{230\text{ nm}}]$ for IDW; then, all of the previously mentioned measures were performed against the correlative PSE concentrations while for direct measurement and CM method for LOR, construction of calibration graphs of LOR was based on plotting the absorbance at 280 nm and 250 nm, conjointly against the correlative LOR concentrations; and then, equations of regression were accurately computed.

Calibration graphs based on amplitude of ratio spectra: for RD and IAM methods For RD method, previously stored spectra of zero-order absorption of PSE were divided by the spectrum of absorption of LOR (30.0 μg/mL); then, the difference between 256.8 nm and 270 nm was recorded. Construction of calibration graph was based on plotting the amplitude difference against

the correlative PSE concentrations while for IAM method, previously stored spectra of zero-order absorption of LOR were divided by the spectrum of absorption of normalized LOR, and the constant value of each spectrum at wavelength region (277–326 nm) was recorded. Construction of calibration graph was based on plotting the constant value against correlative LOR concentrations then equations of regression were accurately computed. For IAM method, previously stored spectra of zero-order absorption of PSE were divided by the absorption spectrum of normalized PSE to get ratio spectrum and the constant value of each at wavelength region (235–250 nm) was recorded. Construction of calibration graph was based on plotting constant value against correlative PSE concentrations; then, equation of regression was accurately computed.

Analysis of prepared mixtures in laboratory

Aliquots were accurately transferred from stock solutions (1500.0 μg/mL) of PSE and (100.0 μg/mL) of LOR. Prepared mixtures in laboratory with various ratios of the mentioned drugs were prepared, and the volume was adjusted till the mark by 0.1 M HCl. Scanning of spectra of previously prepared mixtures was carried out at wavelength region (200–400 nm) and kept at the computer.

Manipulation of the spectra of zero-order absorption of the mixtures

For determination of PSE Absorption correction method (AC): Using the spectra of zero-order absorption of every laboratory previously prepared mixtures, the absorbance was measured at 256.8 nm and 280 nm, and by using the calculated LOR absorption factor, the concentration of PSE in each laboratory previously prepared mixture was obtained using relative equation of regression at 256.8 nm. Dual wavelength method (DW): Using the spectrum of absorption of every laboratory previously prepared mixture, the absorbance was measured at 254 nm and 273 nm. Difference in absorbance was calculated. PSE concentration in every laboratory previously prepared mixture was obtained using the relative equation of regression.

Induced Dual wavelength method (IDW): Using the spectrum of absorption of every laboratory previously prepared mixtures, the absorbance was measured at 263 nm and 230 nm; then, difference using the equality factor of LOR was calculated ($A_{263\,nm}$ − (eq. factor)$_{LOR}$ $A_{230\,nm}$). PSE concentration in every laboratory previously prepared mixture was obtained using the relative equation of regression.

For determination of LOR Direct spectrophotometric determination: Using the spectrum of zero-order absorption of every laboratory previously prepared mixture, the absorbance was measured at 280 nm. The concentration of LOR in each laboratory previously prepared mixture was obtained using the relative equation of regression.

Manipulation of ratio spectra of laboratory previously prepared mixtures using LOR (30.0 μg/mL)

The scanned spectrum of zero-order absorption of every laboratory previously prepared mixture was divided by the spectrum of absorbance of LOR (30.0 μg/mL) to get ratio spectra.

Constant multiplication method paired with spectrum subtraction method (CM-SS) for PSE and LOR The constant of every ratio spectrum of mixture was measured in the wavelength region from (277–326 nm) of each ratio spectrum. The spectrum of zero-order absorption of LOR was obtained through multiplication of the value of constant by the spectrum of the divisor (LOR 30.0 μg/mL). The resolved spectrum of zero-order absorption of PSE (D^0) was obtained after spectrum subtraction of the attained LOR-D^0 from the correlative laboratory previously prepared mixture D^0. The concentration of PSE and LOR in each laboratory previously prepared mixture was obtained using relative equations of regression at 256.8 nm for PSE and 250 nm and 280 nm for LOR.

Ratio difference method for PSE (RD) Using the obtained ratio spectrum of each mixture, the amplitude difference between 256.8 and 270 nm was calculated. The concentration of PSE in every laboratory previously prepared mixture was attained using relative equation of regression.

Manipulation of ratio spectra of previously prepared mixtures by using normalized spectra as a divisor

Induced amplitude modulation method for PSE and LOR (IAM) The ratio spectrum was obtained by division of scanned spectrum of zero-order absorption of every laboratory previously prepared mixture over the spectrum of absorption of normalized LOR, then the constant for each mixture at wavelength region (277–326 nm) was measured, and then subtraction of the value of constant of each mixture from its correlative ratio spectrum was conducted followed by multiplication by the recovery spectrum (computed by dividing normalized spectra of standard LOR by standard PSE) to get constant relative to PSE. The concentrations of PSE and LOR were accurately calculated from their relative equations of regression.

Assessment of pharmaceutical dosage form

Ten tablets of Clarinase® were accurately weighed, the average was computed, and then, they were grinded to

obtain fine powder. An accurate amount equal to one tablet (containing 1200 μg of PSE and 50 μg LOR) were accurately transmitted to 100-mL beaker followed by the addition of 50-mL 0.1 M HCl; then, sonication of the solution was performed using ultrasonic bath for a period of 5 min. The solution was filtered using filter paper (Whatman No.10 filter paper with pore size = 11 μm) into 100-mL volumetric flasks. Using 0.1 M HCl, the volume was adjusted till the mark. Appropriate dilution was performed to get final concentration claimed to contain 240 μg/mL PSE and 10 μg/mL LOR. The proposed methods were applied for the analysis of the studied drugs through the procedures previously cited under analysis of prepared mixtures in laboratory for every method. Using relative equations of regression, PSE and LOR concentrations were calculated.

Results and discussion

The spectra of zero-order absorption of PSE and LOR in 0.1 M HCl show partial overlapping (Fig. 2). The aim of this work is to create simple, accurate, and sensitive methods for concurrent PSE and LOR determination either in their authentic form or their combined pharmaceutical formulation with acceptable precision, besides statistically comparing the proposed methods' ability for the determination the two cited drugs.

Experimentally, different types of solvents such as 0.1 M HCl and methanol were tried; 0.1 M HCl was found to be the best solvent for extraction PSE and LOR from the pharmaceutical dosage form (Clarinase®) and was approved by reported methods [33, 35, 39, 40] without any interference from the excipient satisfactory results for the two drugs PSE and LOR regarding selectivity and precision.

By scanning spectra of the absorption of PSE (at higher concentration ≥ 180.0 μg/mL) and LOR ≥ 5.0 μg/mL in 0.1 M HCl, PSE spectrum shows two maxima at 208.0 nm and 256.8 nm, while LOR has two maxima at 250 nm and 280 nm. Using these higher PSE and LOR concentrations allows the concurrent determination of both cited drugs without LOR enrichment, thus minimizing the error which may be occurred during calculation of claimed LOR. In the region of wavelength of 200–269 nm, severely overlay of spectral bands was observed while LOR spectrum is extended over PSE above this region allowing its direct determination at 280 nm (Fig. 2). For attaining best resolution and quantifying every drug without contribution from the other at wavelength region of 220–300 nm, different spectrophotometric methods were applied, keeping in mind the mentioned linearity for the two cited drugs (180.0–1200.0 μg/mL and 5.0–30.0 μg/mL for PSE and LOR, conjointly) and their ratio in the pharmaceutical dosage form (LOR: PSE, 1:24). Satisfactory results were achieved concerning their bulk powder, laboratory previously prepared mixtures pharmaceutical formulation without needing of initial separation.

Manipulation of spectra of the zero-order absorption of the previously prepared mixtures

For determination of LOR

The spectra of zero-order absorption of each laboratory previously prepared mixture were directly

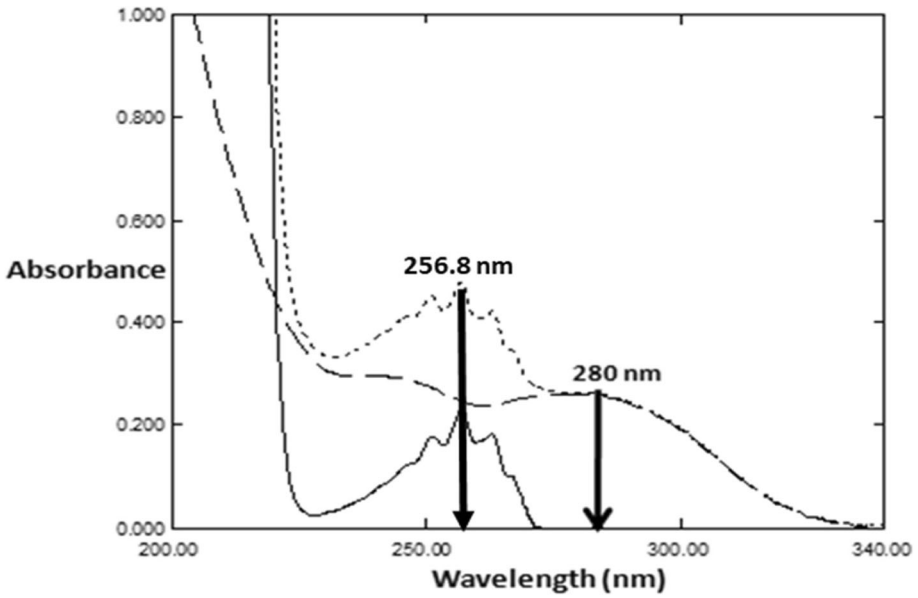

Fig. 2 Zero-order absorption spectra of PSE (s/b a) 240.0 μg/mL, LOR (s/b b) 10.0 μg/mL in a wavelength region (200 – 340 nm) and their binary mixture (s/b c)

measured at 280 nm without any interference of PSE; then, the concentration of LOR in every mixture was determined through its relative regression equation at its maxima 280 nm as shown in Fig. 2.

A linear correlation was obtained between the values of the absorbance of LOR at its λ_{max} 280 nm and the corresponding concentrations (5.0–30.0 μg/mL), and then the regression equation was accurately computed as shown in Table 1.

For determination of PSE

Absorption correction method for PSE (AC) In this method [41, 42], resolution of the absorbance corresponding to PSE in the mixture could be calculated by using the absorption factor of LOR at 256.8 and 280 nm. Recording the spectra of zero-order absorption of PSE and LOR in the range of wavelength 200–340 nm was performed, as shown in Fig. 2. It was found that the two spectra of PSE and LOR are strictly overlapped at (200–275 nm) where PSE has maxima 256.8 nm while LOR shows no interference at 280 nm. The spectra of zero-order absorption of LOR standard stock solutions with various concentrations were recorded at the wavelength region of 200–400 nm. The value of absorption factor was computed which is the ratio between the absorbance at 256.8 nm and 280 nm ($A_{256.8\,nm}/A_{280\,nm}$), and it was found to be 0.952 so the absorbance of PSE in the mixture at 256.8 nm could be calculated after multiplication of this factor by absorbance of mixture at 280 nm ($A_{256.8\,nm}/A_{280\,nm}$) × $A_{280\,nm}$] as shown in Fig. 2. Quantitative estimation of PSE at 256.8 nm in their mixture (LOR + PSE) was carried out by subtracting LOR absorbance value at 256.8 nm from the mixture's recorded absorbance at 256.8 nm, using the following equation:

$$\text{Absorbance of PSE at } 256.8\,\text{nm} = A_{256.8\,nm}(\text{LOR} + \text{PSE}) - [(A_{256.8\,nm}/A_{280\,nm}) \times A_{280\,nm}(\text{LOR} + \text{PSE})].\text{Absorbance of PSE at } 256.8\,\text{nm} = A_{256.8nm}(\text{LOR} + \text{PSE}) - [(0.952) \times A_{280nm}(\text{LOR} + \text{PSE})].$$

where $A_{256.8\,nm}$ (LOR + PSE) and $A_{280\,nm}$ (LOR + PSE) are the absorbance of the mixture at 256.8 nm and 280.0 nm, and $A_{256.8\,nm}/A_{280\,nm}$ is the absorption factor.

The concentration of PSE in each mixture was calculated from its equation of regression which represents linear correlation between the absorbance values of PSE at its λ_{max} 256.8 nm against the relative concentrations (180.0–1200.0 μg/mL), and then, equation of regression was accurately computed as shown in Table 1. This method is restricted for binary mixture analysis in which the drug of interest is extended without any contribution from the interfering drug. This method is advantageous due to its

Table 1 Regression parameters and the results of determination of pure samples of PSE and LOR by the proposed methods

Drug name	PSE					LOR		
methods	D^0 (256.8 nm)(SS)	DW	IDW	RD	IAM	D^0 (280 nm) (Direct measurement or CM)	D^0 (250 nm) (CM)	IAM
Range[a] (μg/mL)	180.00–1200.00					5.00–30.00		
Regressions parameters								
Slope	0.0009	0.0006	0.0006	0.0014	1.0010	0.0260	0.0278	0.9847
Intercept	0.0028	0.0059	0.0184	0.01196	0.1560	0.0019	0.0031	0.0749
Correlation coefficient[b] (*r*)	0.9999	0.9999	0.9999	0.9999	1.0000	0.9999	0.9999	0.9999
Accuracy (mean ± SD)	99.59 ± 0.86	98.51 ± 0.42	99.42 ± 0.76	99.28 ± 0.83	100.04 ± 0.23	101.06 ± 0.49	100.55 ± 0.33	100.88 ± 0.27
Precision (±%RSD)								
Repeatability[c]	0.931	0.508	0.602	0.372	0.142	0.490	0.511	0.518
Inter-day precision[d]	1.159	0.942	1.119	0.775	0.373	0.631	0.714	0.702

[a]Six calibration points in (μg/mL), average of three experiments.
[b]$r = \sqrt{R^2}$
[c]Intra-day precision ($n = 9$), average of 3 different concentrations (200.00, 600.00, and 1000.00 μg/mL) for PSE and (10.00, 20.00, and 25.00 μg/mL) for LOR, repeated 3 times each within the same day
[d]Inter-day precision ($n = 9$), average of 3 different concentrations (200.00, 600.00, and 1000.00 μg/mL) for PSE and (10.00, 20.00, and 25.00 μg/mL) for LOR, repeated 3 times each on 3 successive days

ability to apply on the mixture's zero-order absorption spectrum without needing divisor.

Dual-wavelength method for PSE (DW) The selection of two wavelengths in which the interfering element shows equal value of absorbance, and the desired element differs significantly in the value of the absorbance with concentration is very important factor to apply dual-wavelength method [43, 44]. As selection of suitable wavelengths regarding sensitivity and selectivity is vital, so different wavelengths were examined, and it was found that the best results concerning sensitivity and selectivity were obtained through the absorbance difference at 254 nm and 273 nm for determination of PSE where LOR shows zero absorbance difference (Fig. 3).

In each mixture, PSE concentration was determined through its correlative equation of regression which shows linear correlation between the difference of PSE absorbance at $\Delta A_{254-273\,nm}$) against the corresponding concentrations 180.0–1200.0 μg/mL, and the equation of regression was accurately computed as in Table 1.

The main drawback of this method is that the measurements of absorbance of the drug of interest were performed at critical wavelengths where interfering drug shows zero interference so any deviation in wavelengths leads to high error which will have a negative effect on the robustness of the method. This method is advantageous due to its ability to apply on the mixture's zero-order absorption spectrum without needing divisor or extension of one of the drugs over the other as in AC method.

Induced dual-wavelength method for PSE (IDW) The principle here is abolishing the interfering element's absorbance between two certain wavelengths. On the contrary of the ordinary dual wavelength method (DW), this method is used when the difference in absorbance (ΔA) of interfering element is not equal zero [37].

To determine PSE, LOR absorbance should be abolished at the selected two wavelengths. Therefore, induced dual-wavelength method was performed by calculating pure LOR – equality factor at the two selected wavelengths ($F = [A_{263/}A_{230}] = 0.778$). Upon using this factor, the absorbance of the interfering component (LOR) will equal zero, while the absorbance of the desired component (PSE) will differ at the two selected wavelengths. Calculation of the difference of absorbance of the mixture's zero-order absorption spectrum was performed at 263 nm and 230 nm after multiplication of the absorbance at 230 nm by the equality factor *F*. This calculated difference of absorbance is linked only to PSE, while LOR is removed, as shown in Fig. 4. The ΔA values are replaced in the equation of regression which relate ΔA [$A_{263\,nm}$ – (eq. factor) $A_{230\,nm}$] versus PSE concentration.

In every mixture, PSE concentration was obtained through using its correlative equation of regression which represents the linear relationship between the difference of PSE absorbance at $\Delta A = A_{263\,nm} - 0.778A_{230\,nm}$ and the correlative concentrations 180.0–1200.0 μg/mL; then, the equation of regression was accurately computed as shown in Table 1.

The main benefit of this method is that it has no limitation or requirements as AC method or DW method

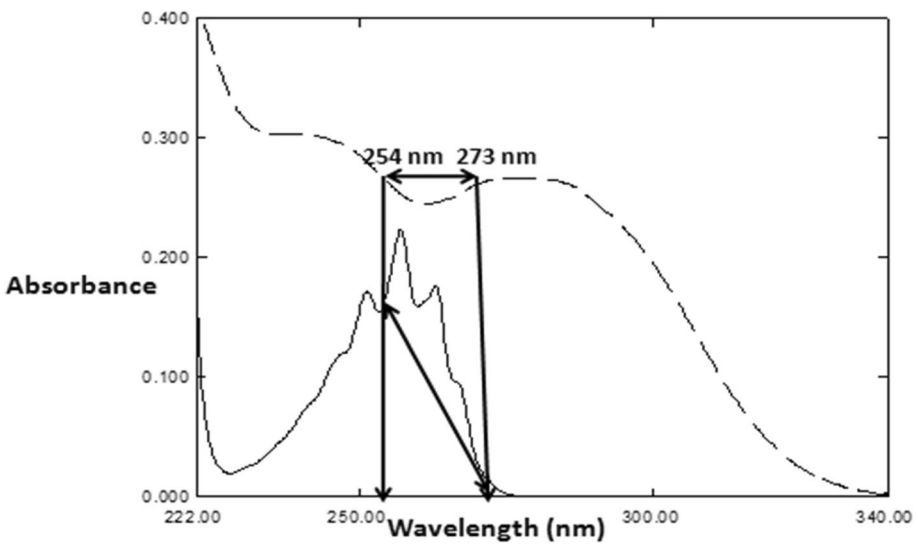

Fig. 3 Zero-order absorption spectra of PSE (s/b d) 240.0 μg/mL and LOR (s/b e) 10.0 μg/mL showing absorbance difference at 254 nm and 273 nm

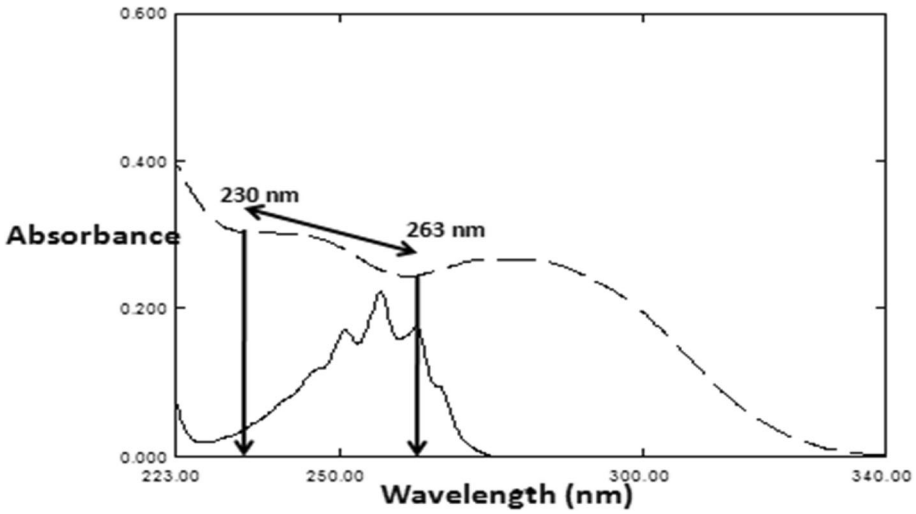

Fig. 4 Zero-order absorption spectra of PSE (s/b f) 240.0 μg/mL and LOR (s/b g) 10.0 μg/mL showing absorbance difference at 230 nm and 263 nm

and it can be widely applied for a variety of binary mixtures.

Manipulation of the ratio spectra of the mixtures using LOR (30.0 μg/mL)

Constant multiplication method paired with spectrum subtraction method (CM-SS)

The ratio spectrum of every laboratory previously prepared mixture could be obtained using a divisor of LOR (30.0 μg/mL); then, the constant (LOR/LOR′) at wavelength region (277–326 nm) was measured (Fig. 5a). By multiplying the divisor by the previously calculated constant, the spectrum of zero-order absorption of LOR in the mixture was determined then concentration of LOR was obtained by using its correlative regression equation at the two maxima 280 nm and 250 nm for LOR (Fig. 5b). This method is advantageous due to its ability to recover the spectra of the zero-order absorption LOR so the resolved spectra which represent spectral profiling of the drug and consequently LOR could be analyzed via their two maxima with best results of accuracy and precision. The resolved spectrum of zero-order absorption of PSE was obtained after subtraction of LOR spectrum (Fig. 5c).

The concentration of LOR in every laboratory previously prepared mixture was determined by using its two correlative regression equations which show linear relation between the values of absorbance of LOR at $\lambda_{250\,nm}$ and $\lambda_{280\,nm}$ and the corresponding concentrations (5.0–30.0 μg/mL) for 280 nm.

In every mixture, PSE concentration was determined through its correlative regression equation which shows linear relation between the absorbance values of PSE at its λ_{max} 256.8 nm and the corresponding concentrations (180.0–1200.0 μg/mL), and then, the regression equation was accurately computed as shown in Table 1.

The main benefit of this method is getting the original spectrum of the two cited drugs; thus, their concentrations could be obtained using their absorbance at λ_{max} with maximum accuracy and precision. In addition, these recovered spectra act as purity index of the proposed drugs.

Ratio difference method (RD) for PSE

In this method, the difference of the amplitude between the two selected wavelengths on the mixture's ratio spectra is directly proportional to the desired element's concentration; it does not depend on of the interfering element [45].

After obtaining the scanned mixture's spectrum followed by its division by LOR divisor absorption spectrum, the ratio spectrum represents $\frac{PSE}{LOR'}$ constant. Selection of two wavelength for PSE ratio spectra and subtraction of these two amplitudes $(\frac{PSE}{LOR})1-(\frac{PSE}{LOR})2$ were performed so the contribution of the divisor element will be omitted while PSE concentration will be relative directly to the difference. The concentration of PSE in each mixture was determined through its relative equation of regression showing linear relation between the amplitude difference of PSE at $(\Delta P_{256.8-270\,nm})$ against the correlative concentrations (180.0–1200.0 μg/mL).

Accurate selection of the two wavelengths and the divisor is very important. The divisor which will be selected should compromise between negligible noise and maximum sensitivity. The prerequisite of the two selected wavelengths is that the drug of interest has

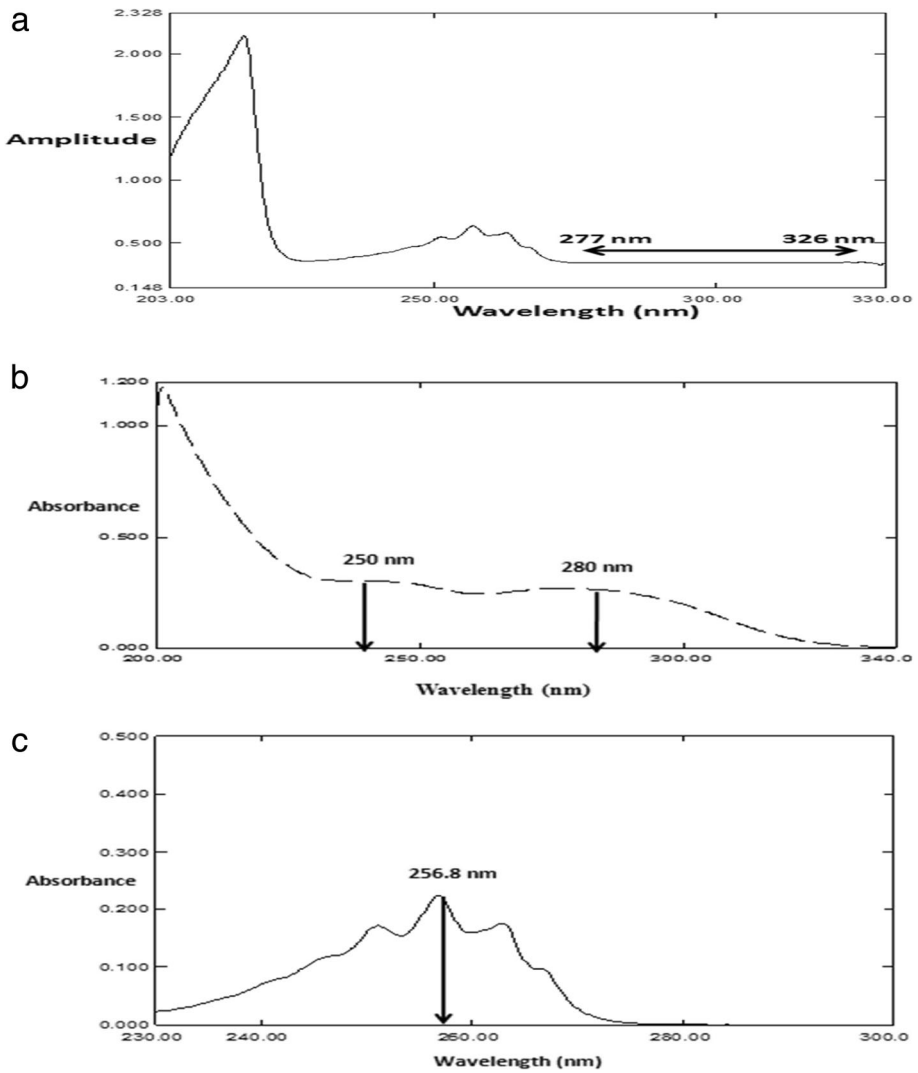

Fig. 5 **a** Ratio spectrum of binary mixture using LOR (30.0 μg/mL) as a divisor and measure the constant at 277–326 nm. **b** The obtained zero-order spectrum of LOR after multiplication by LOR (30.0 μg/mL). **c** The obtained zero-order spectrum of PSE after spectrum subtraction of LOR spectrum

the highest amplitude difference at the contribution region with the interfering substances, so the selected wavelengths were 256.8 and 270.0 nm for determination of PSE in each mixture using LOR divisor (30.0 μg/mL) (Fig. 6) and the regression equation was accurately computed as shown in Table 1.

The most remarkable character of the ratio difference method is simplicity with minimum manipulation steps, no need for calculating of mathematic factor as in AC method and IDW method, maximum accuracy, and reproducibility. The RD method is characterized by the ability to analyze the overlapped spectra without any necessary preliminary steps; in addition, it does not need any sophisticated devices or software.

Manipulation of the ratio spectra of the mixtures using normalized spectra as a divisor

Induced amplitude modulation method for determination of PSE and LOR (IAM)

The recently developed (IAM) method [37] was introduced for the severely overlapped spectra. In this work, the newly developed approach was applied concerned with the partially overlapped spectra where one of the spectra was extended than the other one. The first step of the analysis of the mixture is the division by using the normalized divisor of LOR to get the ratio spectra; then, the constant value was measured at wavelength region [277–326 nm] which represents the concentration of LOR in

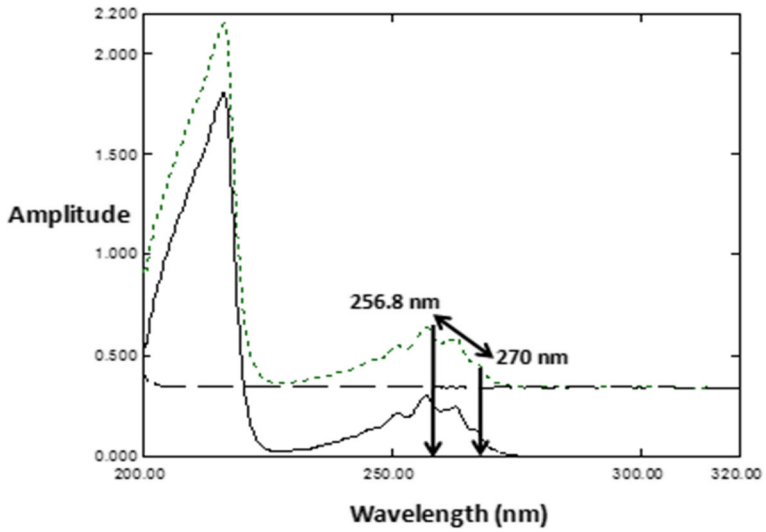

Fig. 6 The amplitude difference at 256.8 nm and 270 nm (ΔP 256.8 -270 nm) of ratio spectrum of binary mixture of PSE and LOR (s/b h), LOR (s/b i) 10.0 μg/mL, and PSE (s/b j) 240.0 μg/mL using LOR (30 μg/mL) as a divisor

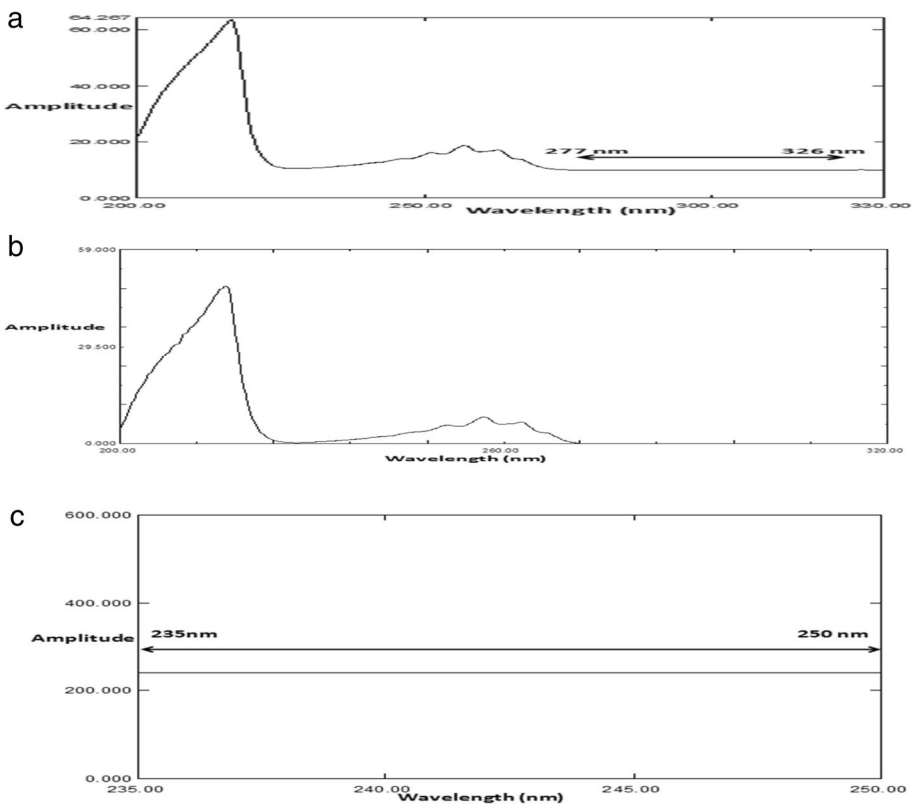

Fig. 7 **a** Ratio spectrum of binary mixture 240.0 μg/mL of PSE and 10.0 μg/mL of LOR using spectrum of normalized LOR′ as a divisor showing the value of constant at (277–326 nm). **b** The obtained ratio spectrum of binary mixture after subtraction of the constant value. **c** The obtained ratio spectrum of binary mixture after multiplication by recovery spectrum (normalized spectrum of LOR/normalized spectrum of PSE) showing the value of constant at 235–250 nm

Table 2 Determination of PSE and LOR in the laboratory-prepared mixtures by the proposed spectrophotometric methods

%Recovery[a] ± SD											
LOR:PSE		PSE						LOR			
Ratio	Concentrations (μg/mL)	IAM	AC (256.8 nm)	DW	IDW	RD	D^0 at 256.8 nm[c] (SS)	IAM	CM		D^0 at 280 nm[d]
									D^0 at 280 nm	D^0 at 250 nm	
(1:24)[b]	10:240	99.43	99.52	100.07	99.83	100.26	101.23	100.58	101.28	99.75	100.20
(1:12)	15:180	99.18	100.57	100.14	99.79	100.13	100.54	100.78	99.24	100.31	100.12
(1:18)	10:180	99.70	99.87	100.12	100.31	100.10	100.34	100.55	100.67	100.07	100.20
(1:30)	20:600	101.15	99.69	99.41	100.09	99.99	101.03	100.64	101.35	100.35	100.84
(1:48)	5:240	99.92	100.76	100.00	100.07	100.02	100.43	99.75	100.77	100.65	99.25
Mean ± SD		99.88 ± 0.76	100.08 ± 0.55	99.95 ± 0.31	100.02 ± 0.21	100.10 ± 0.11	100.71 ± 0.39	100.46 ± 0.41	100.66 ± 0.85	100.23 ± 0.34	100.12 ± 0.57

[a]Average of three determinations
[b]Ratio of the cited drugs as in the dosage form
[c]Direct measurement of PSE at 256.8 nm after spectrum subtraction of LOR
[d]Direct measurement of LOR at 280 nm

the mixture as shown in Fig. 7a. The concentration of PSE was obtained after subtraction of the constant value of LOR from the mixture ratio spectrum (Fig. 7b). The ratio spectrum was obtained which represent $[a_{\mathrm{PSE}}\ c_{\mathrm{PSE}}]/a_{\mathrm{LOR}'}$. Multiplication of the gained ratio spectra by the recovery spectrum representing the absorptivity ratio a_{r} $[(a_{\mathrm{LOR}}/a_{\mathrm{PSE}'})$ obtained by dividing the normalized spectra of LOR by normalized spectra of PSE] was performed and straight line $(a_{\mathrm{PSE}}/a_{\mathrm{PSE}'})$ parallel to X-axis, which represents PSE concentration, as shown in Fig. 7c.

The actual concentrations of PSE and LOR were obtained by direct modulation of their recorded concentrations using the following equations of regression:

$$C_{\mathrm{Recorded}} = 1.001C_{\mathrm{Actual}} + 0.156 \text{ for PSE}$$

$$C_{\mathrm{Recorded}} = 0.9847C_{\mathrm{Actual}} + 0.0749 \text{ for LOR}$$

C_{Recorded} is the amplitude which was recorded for the ratio spectra. It is linked to PSE and LOR concentrations using normalized spectrum of LOR and PSE, conjointly as a divisor, and C_{Actual} is the actual PSE and LOR concentration.

In each mixture, LOR concentration was determined using its correlative regression equation which shows linear relation between peak amplitudes of LOR and the correlative concentrations (5.0–30.0 μg/mL) using normalized LOR′ spectrum as a divisor, while the concentration of PSE in each mixture was determined through using its correlative equation of regression which show linear relation between PSE peak amplitudes and the correlative concentrations (180.0–1200.0 μg/mL) using normalized PSE′ spectrum as a divisor, and the equation of regression was accurately computed as shown in Table 1.

Satisfactory results concerning their pure powdered forms, synthetic mixtures, and the pharmaceutical formulation without needing preliminary separation were obtained.

The IAM method differs from AM method and AAM method as one component is extended than the other and it can analyze mixtures which is lacking the existence of an isoabsorptive point. Also, it can directly modulate both component's ratio amplitudes into their related concentrations. This could be

Table 3 Determination of the studied drugs in the tablet dosage form (Clarinase®) and the application of standard addition technique by the proposed methods

	PSE						LOR			
	IAM	AC	DW	IDW	RD	D^0at 256.8 nm[c] (SS)	IAM	CM		D^0at 280 nm[d]
								At 280 nm	At 250 nm	
Pharmaceutical dosage form[b] (found% ± SD)	100.55 ± 0.15	99.76 ± 0.16	99.40 ± 0.29	99.31 ± 0.16	100.08 ± 0.19	98.74 ± 0.41	99.98 ± 0.11	100.93 ± 0.30	100.32 ± 0.05	100.00 ± 0.21
Standard Addition[a] (recovery% ± SD)	99.91 ± 1.07	99.47 ± 1.27	99.96 ± 0.96	100.19 ± 0.71	100.31 ± 0.68	99.88 ± 0.44	100.37 ± 0.67	101.49 ± 0.54	100.34 ± 1.07	100.71 ± 0.96

[a]Average of five experiments (pure added equivalent to 120, 240, 360, 480, and 600 μg/ml of PSE and 5, 10, 15, 17.5, and 20 μg/ml of LOR)
[b]Average of three experiments
[c]Direct measurement of PSE at 256.8 nm after spectrum subtraction of LOR
[d]Direct measurement of LOR at 280 nm

Table 4 Statistical comparison of the results obtained by the proposed spectrophotometric methods and those obtained by the official ones for the determination of PSE and LOR in their pure powdered forms

Parameters	PSE						LOR			
	D^0 at 256.8 nm (SS)	DW	IDW	RD	IAM	Official method[a]	D^0 (280 nm) (Direct measurement or CM)	D^0 (250 nm) (CM)	IAM	Official method[a]
Mean	100.07	100.12	100.09	99.64	99.72	99.53	100.10	99.82	99.98	100.06
SD	0.69	0.67	0.76	0.65	0.22	0.42	0.68	0.47	0.62	0.75
N	7	7	7	7	7	6	7	7	7	7
Variance	0.4761	0.4489	0.5776	0.4225	0.0484	0.1764	0.4624	0.2209	0.3844	0.5625
Student's *t* test[b]	1.665 (2.201)	1.860 (2.201)	1.601 (2.201)	0.355 (2.201)	1.046 (2.201)		0.104 (2.179)	0.717 (2.179)	0.217 (2.179)	
F test[b]	2.70 (4.95)	2.54 (4.95)	3.27 (4.95)	2.40(4.95)	3.64 (4.39)		1.22 (4.28)	2.55 (4.28)	1.46 (4.28)	

[a]USP methods for LOR is a HPLC method (using C18 column and mixture of dibasic potassium phosphate, methanol, and acetonitrile (7:6:6) as mobile phase with flow rate 1 mL/min), while for PSE is potentiometric titration method (using glacial acetic acid as a solvent and 0.1 N perchloric acid as a titrant) [38]
[b]The figures in parenthesis are the corresponding theoretical values at $P = 0.05$[47]

achieved by using a constant which is parallel along with *X*-axis. The concentrations can be determined easily over the whole *X*-axis, not at definite point.

Method validation

Validation was performed relative to the guidelines of ICH [46] as revealed in Table 1.

Linearity

The linearity of the proposed methods was assessed through analyzing different concentrations of PSE and LOR ranging from 180.0 to 1200.0 μg/ml and 5.0 to 30.0 μg/mL, conjointly. Replication of each concentration was conducted three times. The analysis was performed as per the previously mentioned experimental conditions. Demonstration of linear equations is conducted in Table 1.

Range

The range of the calibration was performed through using the practical range necessary according to Beer's law and the concentration of both PSE and LOR which present in their combined dosage form to give linear, precise, and accurate results (Table 1).

Accuracy

Accuracy was investigated by applying the proposed methods for determination of various samples of PSE and LOR, and the standard addition technique was performed where various known concentrations of pure standard PSE and LOR were added to the pharmaceutical formulation before proceeding the previously mentioned methods. Obtaining the concentrations of both PSE and LOR were performed using the relative equations of regression. Good accuracy of the proposed spectrophotometric methods was obtained. This was shown by the obtained percentages of recovery (Table 1).

Precision

Repeatability and inter-day precision

They could be determined by using three concentrations of PSE and LOR separately; then, they were

Table 5 ANOVA (single factor) for comparison of the results of the proposed spectrophotometric methods and those of the official methods for determination of PSE and LOR in pure powdered forms

Source of variation	Sum of squares	DF	Mean square	*F* value	*P* value	*F* crit
PSE						
Between exp.	3.386712	5	0.67734	1.862518	0.126191	2.485143
Within exp.	12.72846	35	0.36367			
Total	16.11517	40				
LOR						
Between exp.	0.319743	3	0.106581	0.261691	0.852267	3.008787
Within exp.	9.774657	24	0.407277			
Total	10.0944	27				

At the 0.05 level [46]. The population means are not significantly different

examined intra-daily and inter-daily three times on three different days using the proposed spectrophotometric methods. The correlating standard deviations which are correlative to every concentration were computed (Table 1).

Selectivity

Selectivity of the proposed methods was ascertained by the analysis of various synthetic mixtures containing different ratios of cited drugs within the linearity range PSE and LOR within the linearity range. Acceptable results are revealed in Table 2.

Application of the proposed analytical methods in assessment of Clarinase® tablet

The proposed spectrophotometric methods were used for the determination of concentration of both PSE and LOR in their combined dosage form, Clarinase tablet®, and the results are revealed in Table 3, and the validity of the proposed procedures is further evaluated by applying the technique of standard addition showing no interference from excipients. Good percentage recoveries were showed for all the proposed methods, and this allows for their use of regular PSE and LOR analysis in their combined formulation. The obtained results are revealed in Table 3.

Statistical analysis

The comparison of the statistical data obtained by the proposed analytical methods and those by the official USP methods [38] for pure powder showed insignificant differences as revealed in Table 4. To relate the capability of the proposed methods for the determination of PSE and LOR, the results attained by applying the proposed methods were subjected to statistical analysis through one-way ANOVA test for pure powder and all the proposed analytical methods were insignificant differing between each other (Table 5).

Conclusions

In this work, rapid, precise, and accurate spectrophotometric methods were applied for concurrent determination of PSE and LOR in their pure powdered form, prepared mixtures in laboratory, and pharmaceutical formulation through using various manipulation steps for either measurement directly at the spectra of zero-order absorption or to restore their spectra of zero-order absorption.

The validation of the proposed methods was performed using the guidelines of ICH, and satisfactory results were obtained. Those methods could also be conducted in laboratories of the quality control for regular PSE and LOR analysis. The results were subjected to the statistical comparison to each other and also to the official methods of authentic drugs, and insignificant difference was obtained.

Abbreviations

CM: Constant multiplication; DW: Dual wavelength; IAM: Induced amplitude modulation; IDW: Induced dual wavelength; ICH: International Conference on Harmonization; LOR: Loratadine; PSE: Pseudoephedrine sulphate; RD: Ratio difference; USP: United State Pharmacopeia

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Authors' contributions

HL and MA conceived of the study and performed the design of this work. SE carried out the practical work and wrote the manuscript. YF performed the statistical analysis. MA, HL, and YF revised the final manuscript. All authors read and approved the final manuscript.

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