Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 223 (2019) 117322

Contents lists available at ScienceDirect

Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy

journal homepage: www.elsevier.com/locate/saa

Smart Spectral Processing of Data for the estimation of Commonly Used Over-the-counter (OTC) Co-formulated drug; Pseudoephedrine hydrochloride and Ibuprofen

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ARTICLE INFO

Article history:
Received 22 April 2019
Received in revised form 17 June 2019
Accepted 25 June 2019
Available online 27 June 2019

Keywords:
Dual wavelength
Absorbance correction method
Constant multiplication
Constant center
Spectrum subtraction
Pseudoephedrine and ibuprofen

ABSTRACT

Oral pharmaceutical preparation containing pseudoephedrine hydrochloride (PSE) and ibuprofen (IBU) is widely prescribed as over- the- counter (OTC) for treatment of common cold-sinus. Development of four precise and accurate spectrophotometric methods are established for the concurrent determination of (PSE) and (IBU)in this preparation exploiting zero and/or ratio spectra. Method I is a dual wavelength method (DW). method II is a ratio difference method (RD), method III is a constant multiplication coupled with spectrum subtraction method (CM-SS) and method IV is a constant center coupled with spectrum subtraction method (CC-SS). While, absorbance correction method (AC) is successfully established for the determination of (IBU) only exploiting zero order absorption spectra. The calibration curves are linear over the concentration range of 100.0–900.0 μg/mL for (PSE) and 200.0–1000.0 μg/mL for (IBU). No separation steps are required for the spectrophotometric procedures which augments their simplicity. Analyzing synthetic mixtures of the cited drugs evaluated the specificity of the applied methods. Validation of the analysis results have been statistically performed confirming the accuracy and reproducibility of the proposed method through recovery studies which were carried out by following ICH guidelines. Thus, the developed methods can be successfully applied routinely in quality control laboratory.

1. Introduction

Pseudoephedrine HCl (PSE) (Fig. 1a), is chemically (1*S*, 2*S*)-2-methylamino-1-phenylpropan-1-ol hydrochloride. Pseudoephedrine is a sympathomimetic drug belonging to phenylamine and amphetamine chemical classes and used as a nasal/sinus decongestant, as a stimulant [1], or as an antitussive drug [2]. It acts primarily through its direct action on the adrenergic receptor system causing vasoconstriction and relaxation of smooth muscles in the bronchi [3,4]. (PSE) is accessible in many over- the- counter preparations, either as a single ingredient or (more commonly) in combination with antihistamines, guaifenesin, dextromethorphan, and/or paracetamol (acetaminophen) or another NSAID (such as ibuprofen or aspirin). Literature survey revealed that (PSE) has been determined as a single component or in combination with other drugs using spectrophotometric [5], capillary electrophoresis [6,7], HPLC [8–11] and HPTLC [12].

Ibuprofen (IBU) (Fig. 1b), is chemically known as (*RS*)-2-(4-(2-methylpropyl) phenyl) propionic acid. It is a phenyl propionic acid derivative/cyclooxygenase inhibitor from the class of non-steroidal anti-inflammatory drug used in fever and arthritis treatment as an analgesic. Estimation of (IBU) as single formulation was accomplished using spectrophotometric [13], spectrofluorimetric [14], capillary electrophoresis [15], HPTLC [16], and GLC [17]. Also several methods have been described for the estimation of IBU in multicomponent formulation which include spectrophotometric [18], HPLC [19,20], UPLC [21] and HPTLC [22] methods.

Review of the literature designated that there is only one univariate derivative ratio spectrophotometric method existing for this combination's determination. Literature review revealed that both (PSE) and (IBU) in their tablet dosage form have been concurrently estimated viz. multivariate spectrophotometric (chemometric) methods [23], HPLC [24,25] and HPTLC [26] methods.

Coupling of microcomputers with the spectrophotometer made it generally convincing to use mathematical methods to generate derivative and ratio spectra quickly, easily and reproducibly. This expressively enlarged the use of the spectrophotometric technique. The advantages accompanying UV-spectrophotometric technique in saving cost and time if compared to the HPLC technique gave a great chance to gain an important place in pharmacopoeias and the excellent results obtained for accuracy and precision lead to its widespread usage in the analysis of pharmaceutical dosage forms which has increased rapidly in the last few years.

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https://doi.org/10.1016/j.saa.2019.117322
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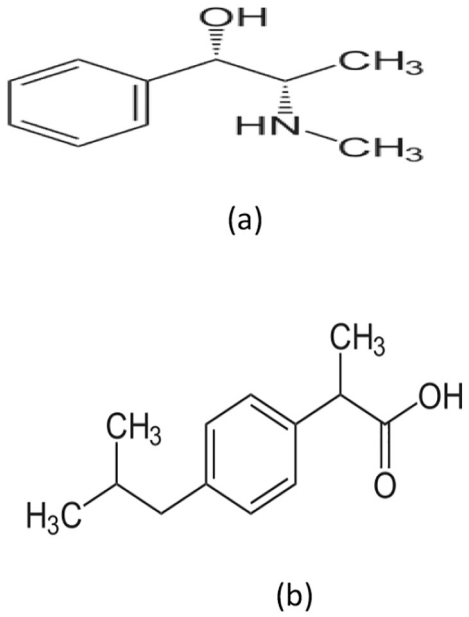

Fig. 1. Structural formula for (a) Pseudoephedrine and (b) Ibuprofen.

Due to the pharmaceutical importance of (PSE) and (IBU) combination as over -the -counter drug (OTC) used for treatment of cold- sinus, this work solicitudes with four simple and selective spectrophotometric methods' development and validation for the proposed drugs' determination in their pure forms and combined dosage form and one spectrophotometric method for the determination of (IBU) only. No derivatization steps were required in the developed methods as the published derivative ratio method thus enhancing signal to noise ratio. Moreover, they can be deemed as cost and time efficacious compared to the published chromatographic methods. On the other hand, complicated procedures of several calibrations set and complicated mathematical or chemometric treatment of data are not required. Although the conventional methods are simple and easy to be applied but they have several limitation and drawbacks. Therefore, this combination was designated for applying newly developed data processing of zero order absorption spectra or ratio spectra using built in spectrophotometer software. The five developed methods were based on utilizing the absorbance difference of the zero order absorption spectra and constant of the ratio spectra without the need to search for zero-crossing points.

The conventional methods' results were contrasted with the recently developed method. Subsequently, conducting a comparative study between the proposed methods and the previously reported one confirmed their effectiveness. Thus, the recently developed spectral data processing was improving their measurement performance without significant costs. The proposed methods have been validated according to ICH guidelines [27] to determine their suitability for the intended use.

2. Theoretical background

The resolution of the binary mixture based on measuring the response of the mixture at two selected wavelengths and by exploiting different data processing, the response of each component is obtained separately then their concentrations are calculated via the corresponding regression equation of each drug.

2.1. Dual wavelength method (DW)

This method [28,29] describes the analysis of two drugs (X) and (Y) in a binary mixture. It is based on ignoring the effect of the interfering drug (Y) at two selected wavelengths where the absorbance difference at these wavelengths was zero. While, the spectra of (X) at these two wavelengths show significant difference which is directly proportional to concentrations of (X).

2.2. Absorbance correction method (AC)

This method [30,31] describes a binary mixture's analysis for two components (X) and (Y) owning partially overlapped spectra with an extension of (Y) over (X). The component (X) shows contribution at wavelength maxima of (Y) (λ_1), while (Y) shows no intervention with (X) at another wavelength (λ_2). For determination of (Y), the value of absorption factor (AF) of pure Y ($A(\lambda_1)/A(\lambda_2)$) is calculated which is the average of proportion of absorbance values (A) of different concentrations of component (Y) at the two selected wavelengths λ_1 and λ_2. Since the absorbance of the mixture (X + Y) at λ_2 corresponds to that of pure (Y) due to absence of contribution of (X) at this wavelength, so the absorbance of (Y) at λ_1 could be calculated using the following equation:

$$\text{Absorbance of (Y) in the mixture at } \lambda_1 = \{A(\lambda_1)/A(\lambda_2)\} \times A\,\lambda_2(X+Y)$$

The concentrations of (Y) will be then calculated from the corresponding regression equation obtained by plotting the absorption values of the zero order spectra, at λ_1 against their corresponding concentrations, respectively.

While, the concentration of (X) could be determined in the mixture by the difference between the absorbance of mixture at λ_1 and absorbance of (Y) in the mixture at λ_1 followed by substitution in its corresponding regression equation.

2.3. Ratio difference method (RD)

Lotfy et al. [32] hosted the ratio difference method (RD) for a binary mixture's analysis (X, Y), where a direct proportionality was illustrated between the amplitude difference of two points on the ratio spectra of a mixture representing component (X) using (Y) as a divisor to the component of interest's concentration (X); independent on the interfering component's concentration (Y). While, component Y can be determined using (X) as a divisor.

2.4. Constant multiplication (CM) coupled with spectrum subtraction (SS) method

Lotfy and Hegazy [33] introduced this method for binary mixture's analysis (X, Y) where, (Y) spectrum is extended over (X). The cited drugs' zero order absorption spectra are attained by recording the constant at the extended area at the selected wavelength region extended within two wavelengths followed by constant multiplication obtaining the zero order absorption spectrum of the extended drug (Y) while the spectrum of the less extended one (X) is obtained by spectrum subtraction of the obtained spectrum from the corresponding laboratory prepared mixture's spectrum. The concentration of each drug is obtained by substitution in the regression equation obtained by plotting the absorbance at their maxima versus the concentrations of each cited drug.

2.5. Constant center (CC) coupled with spectrum subtraction (SS) method

Lotfy [34] presented this method for the analysis of the severely overlapped spectra binary mixture (X, Y). For each mixture, obtaining

the zero order absorption spectrum of (Y) is achieved via ratio spectra of the mixture using (Y) as a divisor by calculating the constant via amplitude difference method at two selected wavelengths followed by constant multiplication while the spectrum of (X) is obtained by spectrum subtraction of the obtained spectrum from the corresponding laboratory prepared mixture's spectrum. The concentration of each drug is obtained by substitution in the regression equation obtained by plotting the absorbance at maxima versus the concentrations of each cited drug.

3. Experimental

3.1. Apparatus and software

Spectrophotometric measurements were carried out on Shimadzu (UV-1800, Japan) double beam spectrophotometer, using matched 1.00 cm quartz cells. Scans were carried out in the range from 200.0 to 400.0 nm at 0.1 nm intervals. Spectra were automatically obtained by Shimadzu UV-Probe 2.43 system software.

3.2. Samples and solvents

3.2.1. Pure samples

A pure sample of pseudoephedrine hydrochloride (PSE) was supplied kindly by the National Organization for Drug Control and Research (NODCAR), Giza, Egypt. Its purity was certified to be 99.33 ± 0.51 according to the reported method [23].

A pure sample of Ibuprofen (IBU) was kindly supplied by Al-Kahira Pharmaceuticals and Chemical Industries, Cairo, Egypt. Its purity was certified to be 99.50 ± 1.35 according to the reported method [23].

3.2.2. Market sample

Brufen-Flu® tablets dosage form, batch number (47115/3J) labelled to contain 30 mg of (PSE) and 200 mg of (IBU), was manufactured by Al Kahira Pharmaceuticals and Chemical Industries, Cairo, Egypt.

3.2.3. Solvents

Purchasing of methanol of spectroscopic analytical grade was done from El-NASR Pharmaceutical Chemicals Co., Cairo, Egypt.

3.3. Standard stock solutions

Standard stock solutions containing 1000.0 μg/mL of (PSE) and 2000.0 μg/mL of (IBU) were separately prepared in methanol.

3.4. Spectral characteristics

The zero order absorption spectra (D^0) of 125.0 μg/mL, 900.0 μg/mL of (PSE) and 500.0 μg/mL, 832.5 μg/mL of (IBU) in methanol were scanned separately over the range of 200.0–300.0 nm.

3.5. Procedure

Different aliquots equivalent to 100.0–900.0 μg/mL for (PSE), and 200.0–1000.0 μg/mL for (IBU), were separately prepared into two separate series of 10-mL volumetric flasks via appropriate dilution of their respective standard stock solutions (1000.0 μg/mL of PSE and 2000.0 μg/mL of IBU) using methanol. The prepared solutions were scanned in the range of 200.0–300.0 nm against methanol as a blank and stored in the computer.

3.5.1. Calibration graphs

3.5.1.1. Methods based on processing data of zero order absorption spectra

3.5.1.1.1. Dual wavelength method (DW). The stored zero order spectral absorbance values were recorded at 258.0 nm and 266.6 nm for (PSE) at 256.0 nm and 263.9 nm for (IBU). Two calibration graphs were plotted relating the difference in absorbance of the stored spectra at 258.0 nm and 266.6 nm for (PSE), 256.0 nm and 263.9 nm for (IBU) against corresponding concentrations, respectively then computing the obtained regression equations of both drugs.

3.5.1.1.2. Absorbance correction method (AC). The absorbance values on the stored spectra of (IBU) were measured at 258.4 nm and 275.0 nm. Construction of a calibration graph was achieved relating the absorbance of IBU's (D^0) spectra at 258.4 nm versus the congruent IBU's concentrations and the regression equation was computed. The absorption factor was calculated which represents the average of the proportional between the two absorbance values of different concentrations of pure (IBU) at 258.4 nm to those at 275.0 nm.

3.5.1.2. Methods based on processing data of ratio of zero order absorption spectra

3.5.1.2.1. Ratio difference method (RD) and Constant center (CC). (PSE) and (IBU) solutions' scanned spectra were divided by the absorption spectra of (IBU) (500.0 μg/mL) and (PSE) (900.0 μg/mL) standard solutions, respectively to obtain the ratio spectra. For (RD) method, the amplitude difference at 251.3 and 272.1 nm for (PSE) and 258.0 and 274.0 nm for (IBU) were plotted against their corresponding concentrations. For (CC) method, calibration graph was constructed relating the amplitude differences of the attained ratio spectra at (251.3 nm and 272.1 nm) against amplitudes at 251.3 nm for (PSE) then computing the regression equations.

Constant multiplication coupled with spectrum subtraction method (CM-SS)- Constant center coupled with spectrum subtraction method (CC-SS): The absorbance of the stored zero order spectra at maxima 258.0 nm for (PSE) and 272.8 nm for (IBU) was plotted versus their corresponding concentrations for the calibration graph's construction then computing the regression equations.

3.5.2. Analysis of laboratory prepared mixtures

Testing the specificity of the proposed spectrophotometric methods was attained by preparing solutions containing diverse ratios of (PSE) and (IBU). The spectra of the prepared mixtures were recorded at (200.0–400.0 nm) and stored in the computer. Then, applying the following data processing.

3.5.2.1. Dual wavelength method (DW). The stored zero order spectra of each laboratory prepared mixture was computed and the absorbance difference was calculated at 258.0 nm and 266.6 nm for (PSE) and at 256.0 nm and 263.9 nm for (IBU) then the concentrations of (PSE) and (IBU) were obtained separately from their corresponding computed regression equations.

3.5.2.2. Absorbance correction method (AC). The absorbance of IBU (A_{IBU}) in each laboratory prepared mixture was calculated using the absorbance of mixture at $\lambda_{258.4}$ ($A_{(IBU+PSE)}$) and absorption factor (AF) of (IBU) then substitute in the following equation:

$$A_{IBU} \text{at } \lambda_{258.4} = AF \times A_{(IBU+PSE)\ \lambda 275.0}$$

where, AF is absorption factor representing the proportional of absorbance of different concentrations of pure (IBU) at 258.4 nm and 275.0 nm.

Then from the corresponding computed regression equation at 258.4 nm, IBU's concentration was calculated.

3.5.2.3. Ratio difference method (RD). Each laboratory prepared mixture's D^0 spectrum was divided by the spectrum of standard (PSE) (900.0 μg/mL) to get its corresponding ratio spectrum then the amplitudes of the ratio spectra were recorded at 258.0 nm and 274.0 nm for (IBU). While, for (PSE) standard (IBU) (500.0 μg/mL) was used as a divisor

and amplitudes at 251.3 nm and 272.1 nm were recorded. Calculation of each drugs' concentration was done by substituting in the computed regression equation using the difference between the recorded amplitudes for each drug.

3.5.2.4. Constant multiplication coupled with spectrum subtraction method (CM-SS). For (IBU), each laboratory prepared mixture's D^0 spectrum was divided by the absorption spectrum of standard solution of (IBU) (500.0 μg/mL) to get its corresponding ratio spectrum. The constant of each mixture representing the amplitude in the plateau region allover two wavelengths 272.0–280.0 nm was recorded then multiplied by the divisor IBU' (500.0 μg/mL) to obtain (IBU's) recovered zero order absorption spectrum. Calculation of (IBU's) concentration in each mixture was done using the corresponding regression equation.

For (PSE), the recovered zero order absorption spectra could be obtained via spectrum subtraction by subtracting the recovered (IBU's) spectra in each mixture from their corresponding mixtures' spectra. Calculation of (PSE's) concentration was done via the congruent regression equation.

3.5.2.5. Constant center coupled with spectrum subtraction method (CC-SS). For IBU, its previously obtained ratio spectrum of each mixture was recorded at [251.3 nm and 272.1 nm]. The postulated amplitude at 251.3 nm was calculated using the corresponding regression equation between the ratio difference versus the corresponding postulated value for the different concentrations of pure (IBU), then after subtracting the mixtures' postulated amplitude from its recorded amplitude to get the constant value of each mixture. The recovered zero order absorption spectrum of (IBU) was multiplied by the spectra of 500.0 μg/mL standard (IBU). The computed regression equation representing (IBU's) absorbance at its maxima was used for calculating its concentration.

Accordingly, (PSE's) D^0 absorption spectra could be attained by spectrum subtraction via subtracting (IBU's) obtained spectra from its corresponding mixtures' spectra. Calculating (PSE's) concentration was done using the corresponding regression equation.

3.5.3. Application to pharmaceutical formulation

Ten Tablets of Brufen- Flu® were weighed and powdered. The average weight was calculated. The powder equivalent to 12.5 mg of (PSE) and 83.25 mg of (IBU) was weighed accurately, transferred into a 100-mL beaker and sonicated with 50.0 mL of methanol. The solution was filtered through Whatman filter paper No. 41 into a 100-mL volumetric flask. The volume was completed with methanol to obtain a solution with final concentration claimed to be 125.0 μg/mL and 832.5 μg/mL of (PSE) and (IBU), respectively. The proposed methods were performed using the procedures stated under each method for laboratory prepared mixture's analysis. Calculation of the cited drugs' concentrations was achieved from their corresponding regression equations for each method.

4. Results and discussion

Today's spectrophotometry is a branch focused on the techniques and methods for processing spectral data via computer manipulations. It is a well-established, but still quickly developing technique differentiating it from many other techniques such as chromatography. This work was designed to develop accurate, precise and reliable spectrophotometric methods for the estimation of (PSE) and (IBU)in their combined dosage Brufen- Flu® tablets.

Spectrophotometric assays for some drugs showed more facilitated, simple and inexpensive methods. Furthermore, it revealed the advantages of low-cost solvents, shorter analysis time and simple instrumentation instead of complex details implemented in the chromatographic method's development. Spectrophotometric analysis exhibited more economic and simple assays either using direct UV determination or by manipulation of the obtained spectra. The applications of cost-effective spectrophotometric methods have renovated the concept of analysis in a highly accurate and precise way.

The zero order spectra of pure drugs were severely overlapped which hinders their determination. The choice of the optimum concentration range depends on the spectral characteristics of the compound, its absorptivity and its ratio in the mixture.

For the effective pharmacological action of this combination, the ratio of (IBU):(PSE) was 6.66:1. Thus, (IBU) represents the major component in the pharmaceutical dosage form. Regarding the compound's spectral characteristics and through scanning the spectra of different concentrations of (PSE) and (IBU), it was observed that both cited drugs exhibited two maxima at two wavelength regions according to their concentrations. The first wavelength region; 200.0–230.0 nm has a high sensitivity and limited for analysis of the targeting drugs in low concentrations with high sensitivity in concentration range 4.0–40.0 μg/mL, 5.0–40.0 μg/mL at 208 nm and 222.4 nm for (PSE) and (IBU), respectively. The main drawback observed at this region that the zero-order absorption spectrum of (PSE) showed noisy peaks with bad linear relationship and poor correlation coefficient; 0.9937 which hinders the analysis of this combination at this wavelength region spectrophotometry. In addition, different orders of derivative spectra of both drugs failed to give satisfactory resolution between the peaks of any cited drug at zero crossing of the other one. Thus, the concurrent determination of any of the studied drugs at wavelength region 200.0–230.0 nm is not applicable and this was agreed with that was stated in the reported derivative ratio spectrophotometric method [23] which was not applied in this wavelength region.

At higher concentrations ranges of the proposed drugs, two maxima at wavelength region 240.0–300.0 nm were observed in concentration range 100.0–900.0 μg/mL, 200.0–1000.0 μg/mL at 258.0 nm and 272.8 nm for (PSE) and (IBU), respectively with an observed obeyance to beer's law and fulfilling the cited drugs' ratio in the dosage form. Upon scanning the spectra of different concentrations of the cited drugs at this wavelength region within their linearity ranges, partially

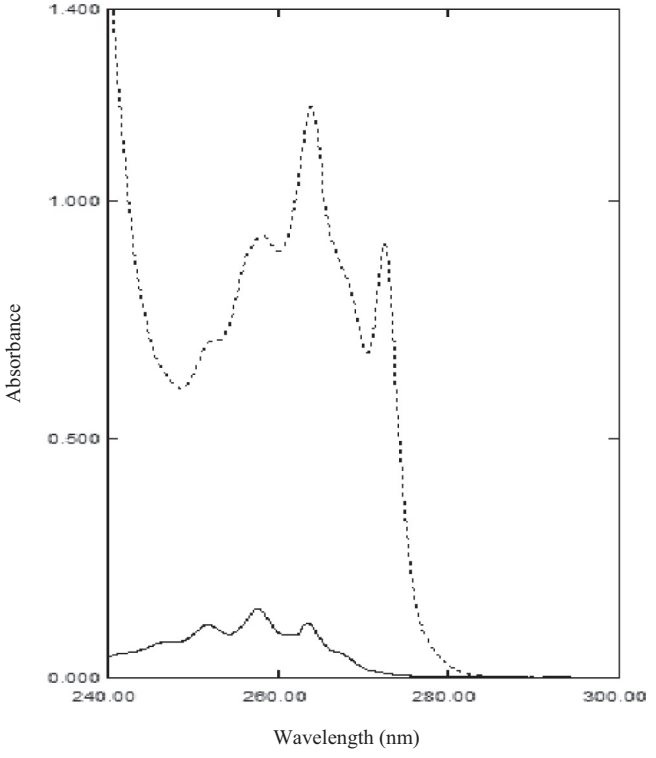

Fig. 2a. Zero-order spectra of 125.0 μg/mL of PSE (—) and 832.5 of μg/mL of IBU (….), separately in the dosage form's ratio.

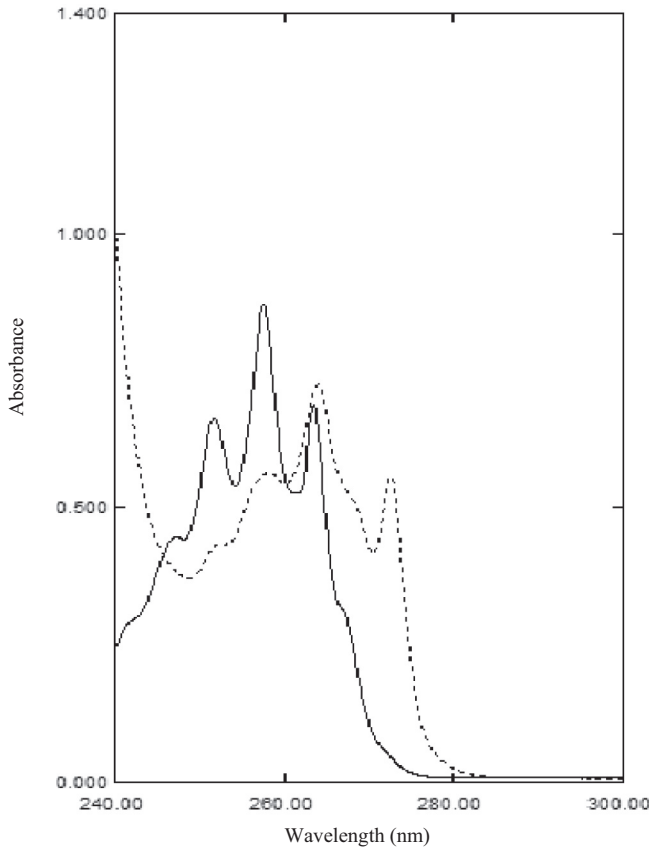

Fig. 2b. Zero-order spectra of 900.0 μg/mL of PSE (—) and 500.0 μg/mL of IBU (....), separately in higher PSE's concentration.

overlapped spectral bands were observed up to 700.0 μg/mL of (PSE) (Fig. 2a). While, upon exceeding this stated concentration for (PSE), the two spectra became severely overlapped (Fig. 2b) and this spectral overlapping either partially or completely hindered the usage of direct UV spectrophotometry for their concurrent determination in a binary mixture.

Spectrophotometric tools and consequently the methods for manipulation of spectrophotometric data are of increasing importance for data processing univariate spectrophotometric methods. Moreover, the development of corresponding applications is driven by a growing demand for this kind of tool to overcome the problem associated with this spectral overlapping between the mentioned drugs in different ratios using zero order absorption spectra or ratio spectra based on the degree of overlapping either partially or completely. For partially overlapping spectra, two methods could be applied, the first one is absorbance correction method based on absorption factor for the interfering drug where the absorbance of the drug of interest can be calculated via simple calculation. The second one is constant multiplication method which utilizes the constant in the extended area and its coupling with spectrum subtraction allows the regain of the D^0 absorption spectra of the proposed drugs in their binary mixtures. While, constant center method overcame the lack of the extension and it resolved this severely overlapped spectra to get the recovered zero order of the proposed drugs. On the other hand, methods as dual wavelength and ratio difference could be easily applied for both spectral overlapping either partially or completely for the determination of (PSE) and (IBU)in their binary mixture. The application of these suggested methods in the higher concentrations' linearity ranges for PSE and IBU enhanced the accuracy of dosage form analysis due to use of large amount of tablet powder so minimize random error due to sample preparation.

4.1. Methods based on processing data of zero order absorption spectra

4.1.1. Dual wavelength method (DW)

The concept of this method is that in the mixture's spectra, a direct proportionality exists between the difference in absorbance between two points and the concentration of component (X); while the absorbance difference is equal to zero for component (Y). For (PSE) determination, 258.0 nm and 266.6 nm were selected where, the difference in absorbance between them is directly proportional to (PSE) concentration opposing a zero-absorbance difference for (IBU) at these wavelengths, (Fig. 3). (PSE) concentration was calculated from the proposed regression equation, (Table 1).

For (IBU) determination, 256.0 nm and 263.9 nm were selected where, the difference in absorbance between them is directly proportional to (IBU) concentration opposing a zero-absorbance difference for (PSE) at these wavelengths, (Fig. 4). (IBU) concentration was then calculated from the proposed regression equation, (Table 1).

The benefit of this method is that the overlapping problem in the original D^0 absorption spectrum is solved without any changes or manipulation steps, thus in turn will save both time and effort, decreases the errors arising from the manipulation steps and sustains maximum accuracy.

The main disadvantage of this method is the restricted choice to the selected wavelengths which are limited to those wavelengths showing the same absorbance of the interfering substance. This necessitates critical measurement of the absorbance of the component of interest, as any minor change in the selected wavelengths will in turn affect the results and consequently will show poor reproducibility and robustness [32].

4.1.2. Absorbance correction method (AC)

(IBU) and (PSE) are found in their dosage form in ratio (6.66:1) where they showed overlapped bands in their zero order absorption spectra but did not show isoabsorptive point (λ_{iso}). For (IBU), the absorption spectra of its standard solutions with different concentrations

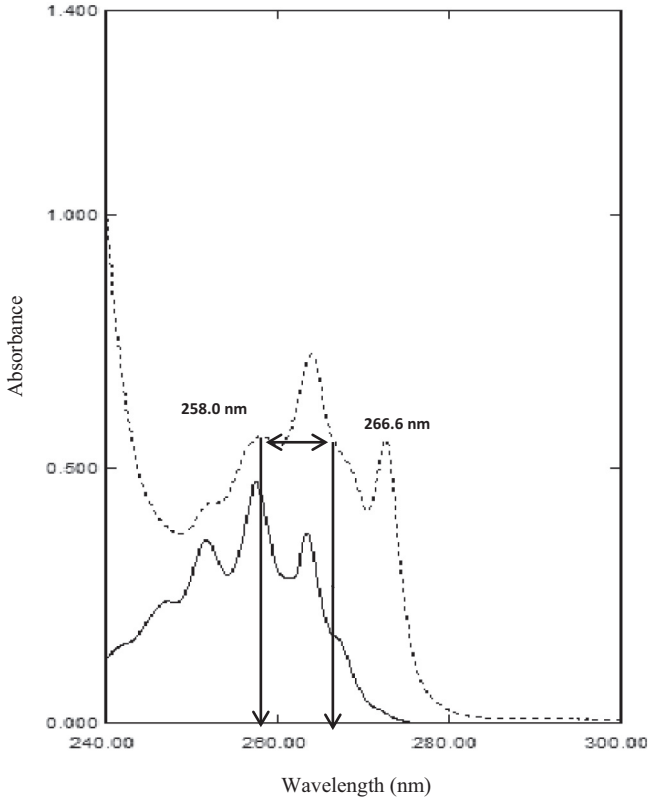

Fig. 3. Zero-order spectra of 500.0 μg/mL of PSE (—) and IBU (....), separately in methanol.

Table 1
Assay parameters and results of determination of pure samples of PSE and IBU by the proposed methods.

Parameter	PSE			IBU			
	DW	RD	CM-SS* CC-SS**	DW	RD	CM-SS* CC-SS**	AC
Range (μg/ml)	100.0–900.0	100.0–900.0	100.0–900.0	200.0–1000.0	200.0–1000.0	200.0–1000.0	200.0–1000.0
Linearity							
Slope	0.0006	0.0016	0.0009	0.0004	0.0264	0.0011	0.0011
Intercept	−0.0015	−0.0098	−0.0110	0.0015	0.4567	0.0049	0.0017
Correlation coefficient (r)	1.0000	0.9999	0.9998	0.9999	1.0000	0.9999	0.9999
n	6	6	6	6	6	6	6
Accuracy							
Mean ± SD	101.18 ± 0.51	100.28 ± 0.74	101.76 ± 1.01	99.63 ± 0.47	100.91 ± 1.07	99.38 ± 0.71	101.39 ± 0.23
Precision							
RSD% [a]	1.185	0.952	0.602	0.966	1.035	1.369	0.654
RSD% [b]	1.520	1.230	1.032	0.993	1.398	1.588	1.008
LOD[c]	15.2	23.6	31.7	23.0	21.0	26.6	31.9
LOQ[d]	46.1	71.8	96.1	69.7	63.8	80.6	96.7

RSD%[a], RSD%[b]: the intra-day & inter-day respectively ($n = 3$) relative standard deviation of concentrations (300.0, 500.0, 700.0 μg/mL for PSE and 400.0, 600.0, 800.0 μg/mL for IBU).
LOD [c] = (S.D of the intercept regression line/slope) × 3.3.
LOQ [d] = (S.D of the intercept regression line/slope) × 10.
* Constant Multiplication coupled with spectrum subtraction.
** Constant center coupled with spectrum subtraction.

were recorded in the wavelength range 240.0–300.0 nm. The absorption factor of pure (IBU) was calculated representing the average of the ratio between absorbance at two wavelengths (258.4 and 275.0 nm)

Absorbance of Ibuprofen (IBU) in the mixture was obtained by multiplying the absorption factor by the absorbance of the mixture at the wavelength where (PSE) showed no contribution (275.0 nm) then (IBU) concentration was calculated using the corresponding regression equation relating the absorbance of (IBU) at 258.4 nm versus its corresponding concentrations, Table 1. Upon applying this method for determination of (PSE) via subtraction the previously calculated absorbance corresponding to (IBU) from the recorded absorbance of mixture to get the absorbance relating of (PSE) in the mixture at 258.4 nm then the concentrations were calculated using computed regression equation at this wavelength. Unfortunately this method fails to give satisfactory results since 258.4 nm isn't suitable for PSE which has a very narrow λ_{max} at 258.0 nm. The constructed regression equation of the zero order spectra of different PSE concentrations at 258.4 nm for PSE shown a poor correlation coefficient < 0.9996 due to bad linearity in

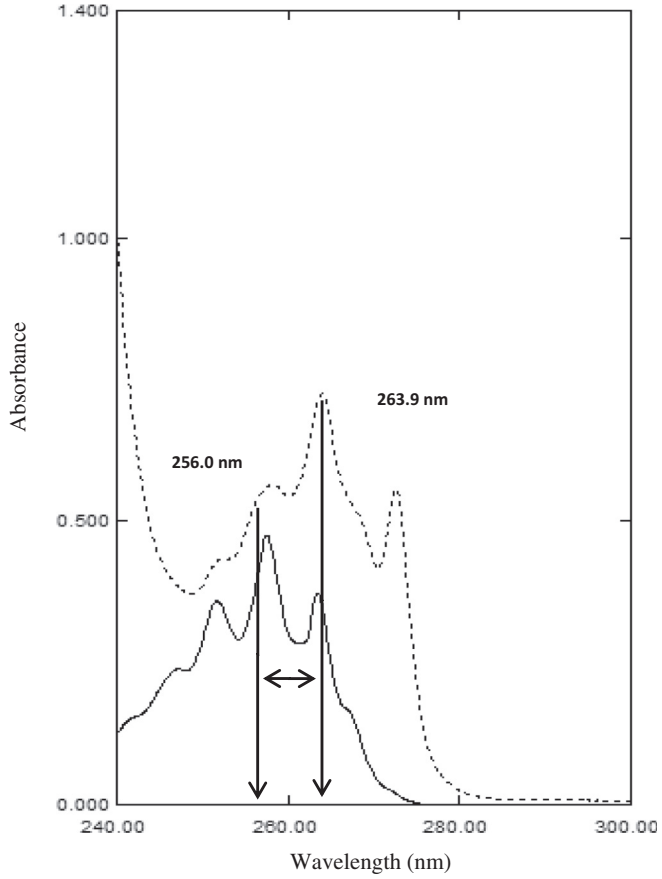

Fig. 4. Zero-order spectra of 500.0 μg/mL of PSE (—) and IBU (….), separately in methanol.

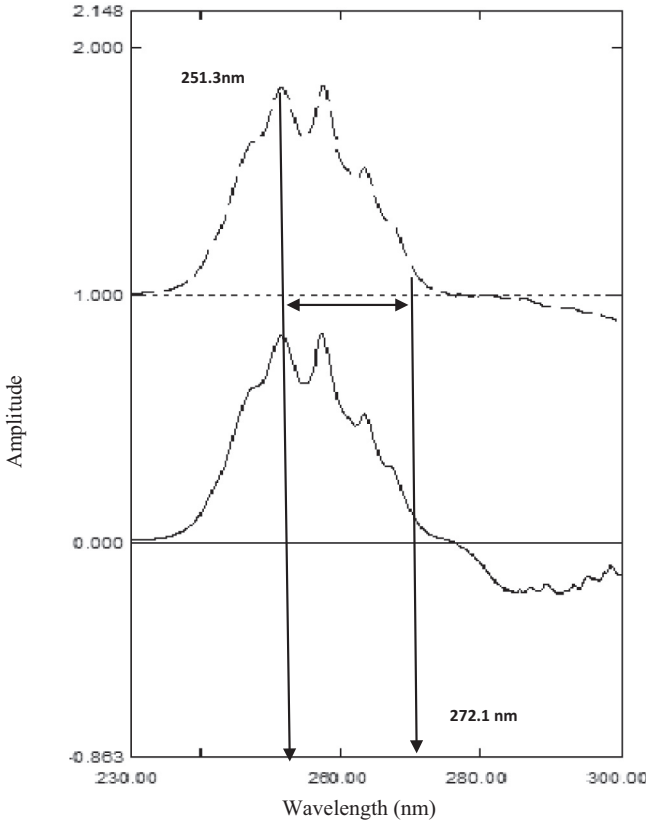

Fig. 5. Ratio spectra of 500.0 μg/mL of PSE (—) and IBU (….) and their binary mixture (——), separately in methanol, using spectrum of IBU (500.0 μg/mL) as a divisor showing the two selected wavelengths (251.3 and 272.1 nm).

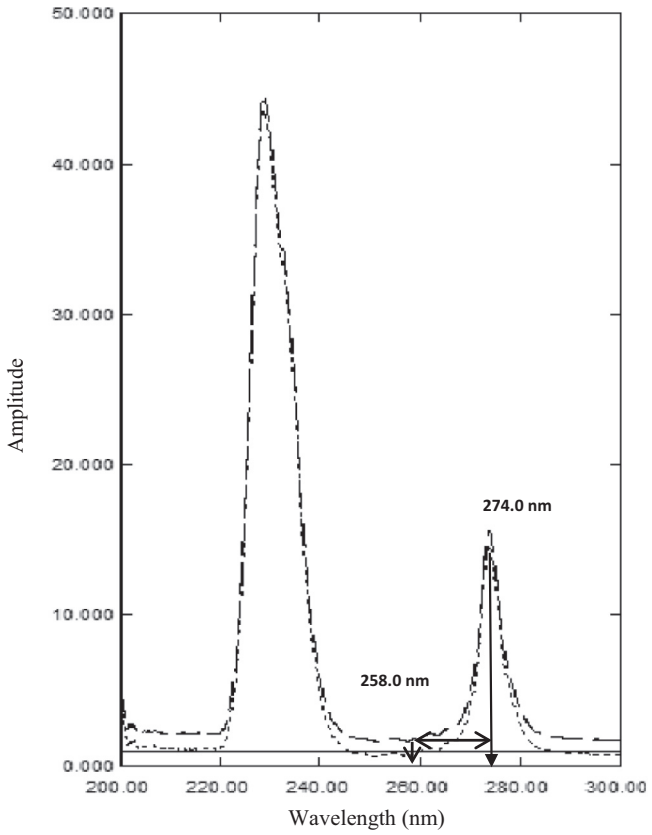

Fig. 6. Ratio spectra of 500.0 μg/mL of PSE (—) and IBU (….) and their binary mixture (——), separately in methanol, using spectrum of PSE (900.0 μg/mL) as a divisor showing the two selected wavelengths (258.0 and 274.0 nm).

the shoulder regions and consequently bad recoveries percentages of the PSE concentrations in the mixtures were obtained.

This main drawback restricted the application of absorbance correction method for simultaneous determination of binary mixtures which their zero order absorption spectra have maxima at the same wavelength but with different absorptivities.

4.2. Methods based on processing data of ratio of zero order absorption spectra

4.2.1. Ratio difference method (RD)

This method is simple, accurate, economic and time saving with few applied manipulation steps. (RD) method is capable of solving the severely overlapped spectra efficiently with neither former separation nor the usage of any complicated apparatus or particular computer programs.

(RD) method was applied to solve the problem of the overlapped absorption spectra of the PSE and IBU in their mixture. The chief principle of the (RD) spectrophotometry is that the scanned mixture's absorption spectrum was divided by the absorption spectrum of the standard solution of interfering component obtaining the corresponding ratio spectrum of drug of interest which represents $\frac{PSE}{IBU}+$ constant or $\frac{PSE}{IBU}+$ constant. Thus, the amplitude difference at two selected wavelengths will representing $(\frac{PSE}{IBU})1\text{-}(\frac{PSE}{IBU})2$ or $(\frac{IBU}{PSE})1\text{-}(\frac{IBU}{PSE})2$, cancelling the interfering component.

The two main factors which affect the ratio difference method are the divisor's choice and the selection of the two wavelengths. A study was carried out to examine the effect of divisor concentration on the ratio spectra since the amplitude of peaks of the ratio spectra is increased or decreased upon changing divisor concentration; however, the positions of the peaks remain unaffected by changing the divisor concentration. The selected divisor should compromise between minimal noise and maximum sensitivity. The only obligation in selecting the wavelength is that the interfering substance should possess spectral contribution at the two selected wavelengths. So, the amplitudes at 251.3 and 272.1 nm were selected for (PSE) determination in the mixture using (IBU) (500.0 μg/mL) as a divisor (Fig. 5). Similarly, the amplitudes at 258.0 and 274.0 nm were selected for (IBU) estimation in the mixture using (PSE) (900.0 μg/mL) as a divisor (Fig. 6).

The advantages of this method over the dual wavelength method that the interfering component is constant which is a straight line all over the curve thus the difference at any two wavelengths will be equal to zero where the drug of interest shows highest amplitude difference leading to higher sensitivity as well as highly reproducible and robust results without the need for critical measurements at certain wavelengths.

4.2.2. Constant multiplication coupled with spectrum subtraction method (CM-SS)

In this work, by dividing each laboratory prepared mixture's D^0 spectrum by a D^0 spectrum of standard (IBU) (500.0 μg/mL) as a divisor spectrum to get its corresponding ratio spectrum.The constant value of each mixture was measured from the straight line parallel to the wavelength axis at the extended part (272.0–280.0 nm) followed by multiplication of this constant by the divisor spectrum to get the recovered spectrum D^0 curve of (IBU)in the mixture. The concentration of (IBU) could be calculated by substitution in its regression equation representing the linear relationship between the absorbance of the zero order spectra at its λ_{max} 272.8 nm against IBU's corresponding concentrations. Table 1.

Table 2
Determination of the studied drugs in laboratory prepared mixtures and in tablet dosage form by the proposed methods.

Sample	Pseudoephedrine ([a]mean ± SD %)				
Method	DW	RD	CM-SS	CC-SS	AC
Laboratory-prepared mixtures ($n = 6$)[b]	99.71 ± 1.59	99.60 ± 1.69	100.25 ± 1.51	98.71 ± 0.88	–
Brufen-Flu® Batch No.47115/3J	100.45 ± 0.60	98.24 ± 0.43	100.23 ± 0.45	97.24 ± 0.67	–
Sample	Ibuprofen ([a]mean ± SD %)				
Method	DW	RD	CM-SS	CC-SS	AC
Laboratory-prepared mixtures ($n = 6$)[b]	99.88 ± 0.92	99.08 ± 1.04	101.16 ± 0.45	101.11 ± 0.70	100.31 ± 0.81
Brufen-Flu® Batch No.47115/3J	91.13 ± 0.31	97.29 ± 0.61	98.15 ± 0.32	97.74 ± 0.65	101.77 ± 0.71

[a] Mean ± SD.
[b] 6 sets foreach method, each of 3 replicates.

PSE's recovered zero order absorption spectra were then obtained by subtracting the recovered IBU's spectra from their corresponding laboratory prepared mixtures. Calculating PSE's concentration was done using the corresponding regression equation representing the linear relationship between the absorbance of PSE's zero order spectra at its λ_{max} 258.0 nm against its corresponding concentrations. Table 1.

This applied method gave satisfactory results for mixtures containing up to 700.0 μg/mL (PSE) where (IBU) has an extended area than PSE and their spectra are partially overlapped (Fig. 5).

4.2.3. Constant center coupled with spectrum subtraction method (CC-SS)

Constant center coupled with spectrum subtraction spectrophotometric method was implemented for resolving spectral overlapped mixtures as (PSE) and (IBU)in the presence of high concentration of (PSE) exceeding 700.0 μg/mL. Thus, the absorption spectra of (PSE) and (IBU) showed severe overlapping with no extension of (IBU) that hinders the application of constant multiplication method coupled with spectrum subtraction method.

In this method, the zero-order absorption spectrum of the mixture (PSE+ IBU) was scanned and divided by the absorption spectrum of 500.0 μg/mL of (IBU') as a divisor obtaining a ratio spectrum represents {(PSE/IBU') + constant}. Ratio difference at two selected wavelength (251.3 nm (λ_1) and 272.1 nm (λ_2)) was calculated {$(PSE/IBU')_1 - (PSE/IBU')_2$}, cancelling the interfering substance (IBU) and subsequently showing no interference.

The practical ratio amplitude at 251.3 nm was recorded for each laboratory prepared mixture; {(PSE/IBU') + (IBU/IBU')}. Calculation of the postulated ratio amplitude value of (PSE/IBU') was attained using the equation representing the linear relationship between the ratio difference of ratio spectra at 251.3 nm and 272.1 nm against the corresponding ratio amplitudes at 251.3 nm.

$$P_1 - P_2\ (\Delta P) = 0.9233P_1 + 0.0144\ (r = 1)$$

where; P_1, P_2 are the ratio amplitudes at 251.3 nm and 272.1 nm of the ratio spectra of different concentration of (PSE) (100.0–900.0 μg/mL) using 500.0 μg/mL (IBU) as a divisor.

The constant value (C·V.) was calculated by measuring the recorded and postulated amplitudes' difference at 251.3 nm for PSE; $[P_{recorded}]$ - $[P_{postulated}]$.

$C.V = [P_{recorded}]$ - $[P_{postulated}]$. Where; C. V is the constant value, $P_{recorded}$ is the recorded amplitude of the ratio spectrum of the laboratory prepared mixture using 500.0 μg/mL (IBU') as a divisor at 251.3 nm and $P_{postulated}$ is the calculated amplitude using the specified regression equation.

By multiplying the obtained constant (IBU/IBU') of the laboratory mixture by (IBU') (Y') (the divisor), the recovered D^0 absorption spectrum of IBU (Y) could be attained which is used for direct determination of (IBU) at 272.8 nm. IBU's concentration was calculated from the corresponding regression equation attained by plotting the absorbance values of the zero order spectra at its λ_{max} 272.8 nm against IBU's corresponding concentrations, Table 1.

PSE's recovered zero order absorption spectrum (D^0) can be determined by subtracting the obtained spectra of (IBU) from their laboratory prepared mixtures' corresponding spectra. The concentration of (PSE) was obtained using the corresponding regression equation, Table 1.

Coupling of spectrum subtraction method with constant center method has the benefit of obtaining the zero-order absorption spectrum of both drugs acting as spectral profile of the drug subsequently the drugs were analyzed by using the absorbance value at their λ_{max} which offered maximum accuracy and precision. In addition, this method offered the opportunity for resolving the spectra of the two proposed drugs even in high concentrations of them. Thus, obtaining the concentrations of both drugs through minimum manipulation steps using only one of the components as a divisor with no need to perform and repeat the same steps for the other drug.

Table 1 represents the statistical parameters of the regression equations and the concentration ranges for all the proposed methods for the determination of pure drugs where satisfactory results were attained.

Table 3
Statistical analysis of the proposed methods and the reported method of PSE and IBU in their pure powdered form.

Parameter	DW	RD	CM-SS CC-SS	AC	Reported method[a] [23]
	Pseudoephedrine				
Mean	101.60	100.73	101.20		99.33
SD	0.48	0.84	0.81		0.51
n	6	6	6		6
Variance	0.2304	0.7056	0.6561		0.2601
t-test *(2.228)	0.008	0.006	0.001		
F *(5.05)	1.12	2.71	2.52		
	Ibuprofen				
Mean	98.36	99.84	97.75	100.53	99.50
SD	0.53	0.56	0.53	0.59	1.18
n	6	6	6	6	6
Variance	0.2809	0.3136	0.2809	0.3481	1.3924
t-test *(2.228)	0.066	0.609	0.013	0.119	
F *(5.05)	4.95	4.44	4.95	4.00	

* The figures in parenthesis are the corresponding theoretical values at $P = 0.05$.
[a] The reported method used is the derivative ratio spectrophotometry at 252.2 nm for PSE and 254.2 nm for IBU, using methanol:0.1 M HCl (3:1) as a blank.

5. Method validation

The evaluation of the methods should be considered during the method development phase. In fact, the process of validating methods cannot be separated from the actual development of the methods conditions, since it is not possible to know whether the method conditions are acceptable until validation studies are performed. The development and validation of new analytical methods may therefore be an interactive process.

Validation of the proposed spectrophotometric methods was accomplished according to the ICH guidelines [27] with respect to methods' sensitivity, linearity range, accuracy and precision as shown in Table 1 showing that both CM-SS and CC-SS have the same results for each proposed drug respectively, and this is because both methods regained the zero order absorption spectra of the studied drugs. Thus, the concentration of each drug was calculated using calibration curves

Table 4
Statistical analysis of the proposed methods and the reported method of PSE and IBU in their dosage form.

Parameter	DW	RD	CM-SS	CC-SS	AC	Reported method[a] [23]
	Pseudoephedrine					
Mean	100.45	98.24	100.23	97.24		90.26
SD	0.60	0.43	0.45	0.67		0.55
n	4	4	4	4		4
Variance	0.3600	0.1849	0.2025	0.4489		0.3025
t-test*(2.447)	0.001	0.010	0.003	0.019		
F*(6.09)	1.19	1.63	1.49	1.48		
	Ibuprofen					
Mean	91.13	97.29	98.15	97.74	101.77	103.45
SD	0.31	0.61	0.32	0.65	0.71	0.50
n	4	4	4	4	4	4
Variance	0.0961	0.3721	0.1024	0.4225	0.5041	0.2500
t-test*(2.447)	0.003	0.015	0.040	0.025	0.055	
F*(6.09)	2.60	1.48	2.44	1.69	2.01	

* The figures in parenthesis are the corresponding theoretical values at P = 0.05.
[a] The reported method used is the derivative ratio spectrophotometry at 252.2 nm for PSE and 254.2 nm for IBU, using methanol:0.1 M HCl (3:1) as a blank.

Table 5
Results of ANOVA (single factor) for the comparison of the proposed methods and the reported method of PSE and IBU in their pure form.

Source of variation	SS	df	MS	F	P-value	F crit
Pseudoephedrine						
Between groups	2.39519	4	0.479038	0.388563	0.851697	2.620654148
Within groups	29.58828	24	1.232845			
Total	31.98347	28				
Ibuprofen						
Between groups	11.17572	5	2.235144	1.486419	0.223658	2.533555
Within groups	45.11133	30	1.503711			
Total	56.28706	35				

representing absorbance at its maxima versus their corresponding concentrations. Table 2 shows the results obtained from the analysis of laboratory prepared mixtures containing diverse ratios of the drugs ensuring the specificity of the proposed methods where satisfactory results were obtained over the calibration range. The proposed methods were also functioned for the determination of the drugs in Brufen-Flu® tablets where satisfactory results were obtained as shown in Table 2.

6. Statistical analysis

Tables 3 and 4 showed statistical comparison of the results obtained by both proposed methods and the reported spectrophotometric method for the cited drugs in their both pure and pharmaceutical dosage forms, respectively. The calculated *t* and F values were found to be less than the theoretical ones demonstrating that there was no considerable difference between the proposed and reported method regarding both accuracy and precision.

Statistical analysis using one way ANOVA test was also performed on the results obtained by applying the proposed methods and those obtained by the reported method, where calculated F (F_{cal}) values were always less than tabulated F (F_{tab}) values for both studied drugs proving that there is no significant difference between the proposed methods and the reported one, Table 5.

7. Conclusion

Simultaneous analysis of binary mixture of (PSE) and (IBU) was applied using smart and simple developed spectrophotometric methods. The proposed methods were simple, accurate, precise, do not need any sophisticated apparatus or a special program. Absorbance correction (AC) and constant multiplication coupled with spectrum subtraction (CM-SS) methods were restricted for the analysis of mixtures containing ≤700.0 μg/mL (PSE). The drawback of the critical measurement in dual wavelength method (DW) made the ratio difference (RD) and constant center coupled with spectrum subtraction (CC-SS) methods superior over it in the determination of both drugs in different ratios. Constant center coupled with spectrum subtraction method (CC-SS) considered being the best method since it succeeded to resolve the spectral overlapping between (PSE) and (IBU) in different ratios and regaining their zero order absorption spectra which acts as fingerprint as well as purity index for the cited drugs. The proposed methods could be easily applied in quality control laboratories as they are having equal accuracy and precision compared to the reported method; in contrast they are of lower cost. Accordingly, the proposed methods could be successfully applied for routine analysis of the studied drugs either in their pure bulk powders or in dosage form in quality control laboratories lacking liquid chromatographic instruments with no preparatory separation steps.

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