Research article

New spectral resolution techniques for resolving and determining the components in binary fixed-dose combinations

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ABSTRACT

Four spectrophotometric approaches were performed to determine a binary combination of Phenazone and Benzocaine in pure powder form and in pharmaceutical formations. This investigation submits the application of four techniques contingent on the presence of the extended area of the spectra of one compound in the binary mixture, these methods include Absorptivity Centering (a-centering), Absorbance Subtraction (AS), Amplitude Modulation (AM) and Concentration Value (CV). The linearity range for the above-mentioned approaches was found to be 3.0–15.0 μg/mL for Benzocaine in a-centering method and 3.0–30.0 μg/mL for Benzocaine and Phenazone in other advanced methods. The four techniques were evaluated as per ICH criteria and were successfully utilized for the determination of Phenazone and Benzocaine existing in pharmaceutical formulations. All results gained by the submitted approaches were statistically compared with a previously published method, and no important differences were detected.

1. Introduction

In this investigation, four spectrophotometric approaches were used for the simultaneous quantification of binary drug combination, containing two partial overlapped spectra where one of them has extended area than the other. The presence of this more extended area of the spectrum has the advantage that no interference from the less extended one, which permits the simultaneous quantification of the binary drug combination. Phenazone and Benzocaine were chosen for this study since they are the best spectrum model for applying these spectrophotometric methods; where Benzocaine spectrum is extended over Phenazone, and Phenazone does not show any contribution at another wavelength.

Phenazone PHN (1,2-Dihydro-1,5-dimethyl-2-PHNnyl-3H-pyrazol-3-one) Fig. 1, has also another name antipyrine which is considered as analgesics [1], while Benzocaine BEN Fig. 1 (ethyl 4-aminobenzoat) is used as topical anesthetics [1]. Various approaches were announced for PHN determination like spectrophotometry [2, 3, 4, 5] HPLC [6, 7, 8], TLC [9], GC [10, 11] and capillary zone electrophoresis methods [12], whereas BEN was determined by spectrophotometry [13, 14, 15, 16, 17, 18], HPLC methods [19, 20, 21, 22, 23, 24]. Simultaneous quantification of PHN and BEN existing in otic drop formulation was announced in previous published studies by spectrophotometric [25, 26], HPLC [27,

28] and TLC [29] methods.

Fortunately, the last period has shown notable growth in the intelligent spectrophotometric techniques in the design and practice fields. In addition, their recent application to phase 1 drug development, where they are used to determine the solubility of new drug through the development process [30]. In this paper, the study of the effectiveness of four spectrophotometric techniques lately developed, based on the extended spectral region, was carried out.

These spectral methods are: Absorptivity Centering (a-centering), Absorbance Subtraction (AS), Amplitude Modulation (AM) and Concentration Value (CV).

Absorptivity Centering (a-centering) method was recently discovered and applied for the determination of two components (X and Y) representing an iso-absorptive point crossing with partly or totally overlapping spectra. through this technique the zero order spectrum of the two components could be obtained and as a result a spectral profile and purity of the peak of each component were achieved [31, 32, 33, 34].

Absorbance Subtraction (AS) technique was also discovered to be helpful in estimating drugs in their mixtures, allowing the two components to be determined in their mixture without the need of a supplementary method, this technique required the presenting of iso-absorptive point as well as mathematically calculating of the factor corresponding to
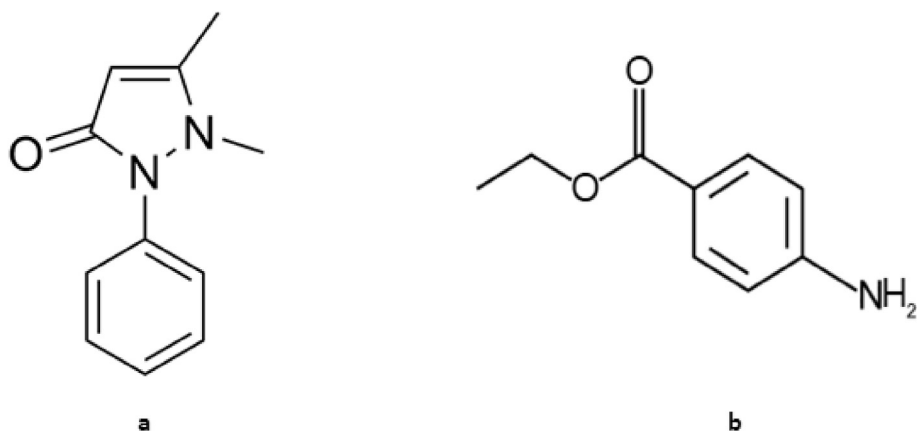
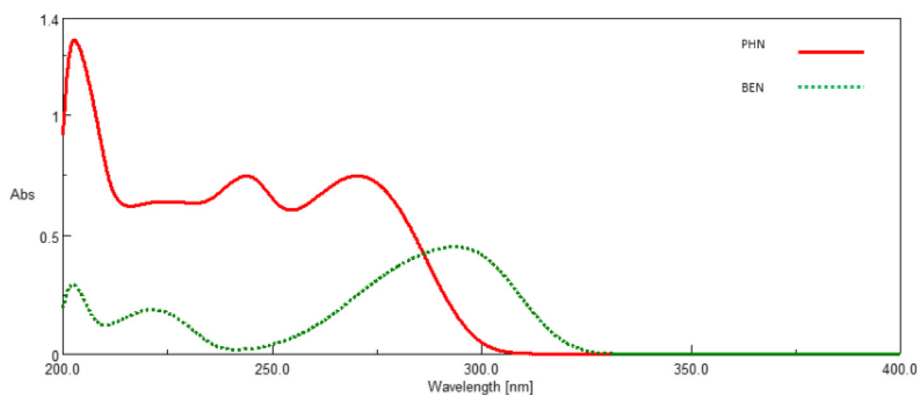**Fig. 1.** Structural formulae of (a) Phenazone and (b) Benzocaine.

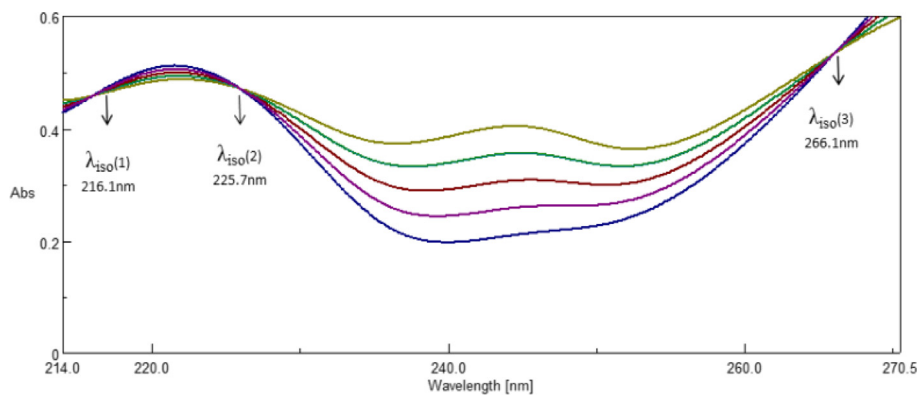**Fig. 2.** Zero-order spectra of [PHN] 13.5 μg/mL and [BEN] 3.5 μg/mL in ethanol.

Fig. 3. Zero-order spectra of mixtures of PHN + BEN, with a total concentration equal to 10.0 μg/mL showing the three iso-absorptive points.

the more extended part spectrum [35].

Amplitude Modulation (AM) method was developed for the analysis of two drugs in their binary mixture via one regression equation. To apply this technique, the presenting of isosbestic point is required as well as the normalized spectra should be obtained [35, 36, 37, 38].

Concentration Value (CV) method is a new strategy based on the graphical display of the spectra, where the drug's concentration value is registered directly on the spectral graph reflecting the real concentration without replacing it in the regression equation. However, this strategy needs preparation of normalized spectrum as well as another spectral technique which should be connected with, in order to complete the simultaneous determination of the components in mixtures [39, 40, 41].

These previously methods were suggested to determine PHN and BEN in pure form as well as in tympanil®, which is an otic pharmaceutical formulation recommended to relieve pain and reduce inflammation in acute otitis media. Furthermore, statistically studies were carried out through the presented methods and the previously published methods [26] where insignificant differences were detected, as well as the efficacy of the suggested techniques was verified by carrying out a comparative review with the previously UV published method. The submitted approaches were validated in relation to (ICH) criteria and were shown to be accurate, precision and selective [42].
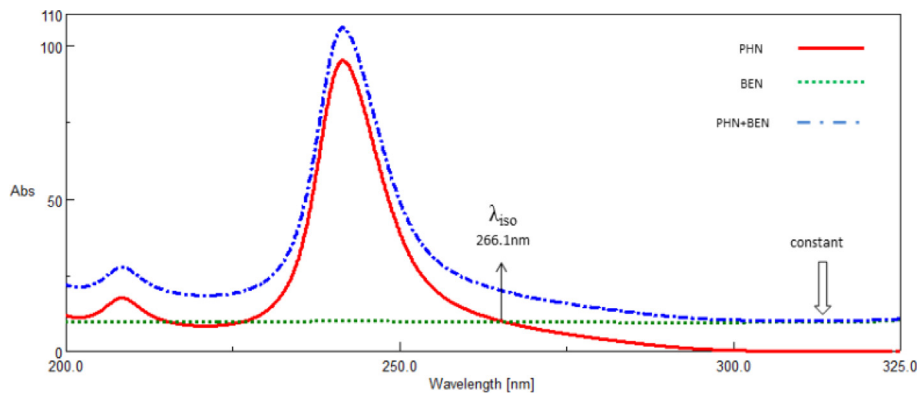
Fig. 4. Ratio spectra of 10.0 μg/mL of PHN, BEN separately in ethanol and their binary mixture,10.0 μg/mL of each in ethanol. using the (NS′ $_{BEN}$) as a divisor showing the constant region.
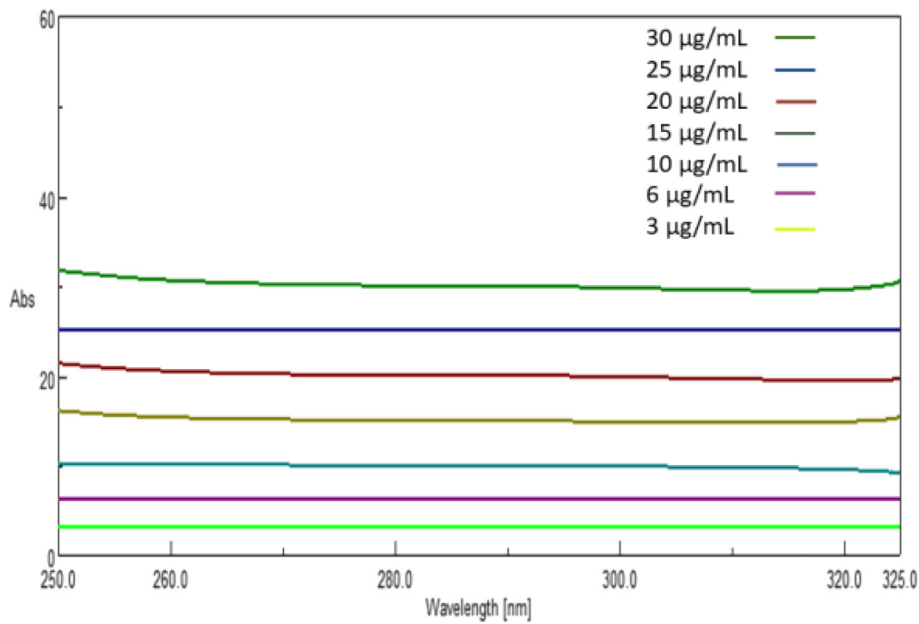
Fig. 5. The constant value gained by dividing the zero order spectra of [BEN] (3.0–30.0 μg/mL) by the normalized spectra (1.0 μg/mL of BEN).

Fig. 6. The constant value gained by dividing the zero order spectra of [PHN] (3.0–30.0 μg/mL) by the normalized spectra (1.0 μg/mL of PHN).

Table 1

Assay parameters and approaches' validation achieved by applying the proposed spectrophotometric approaches.
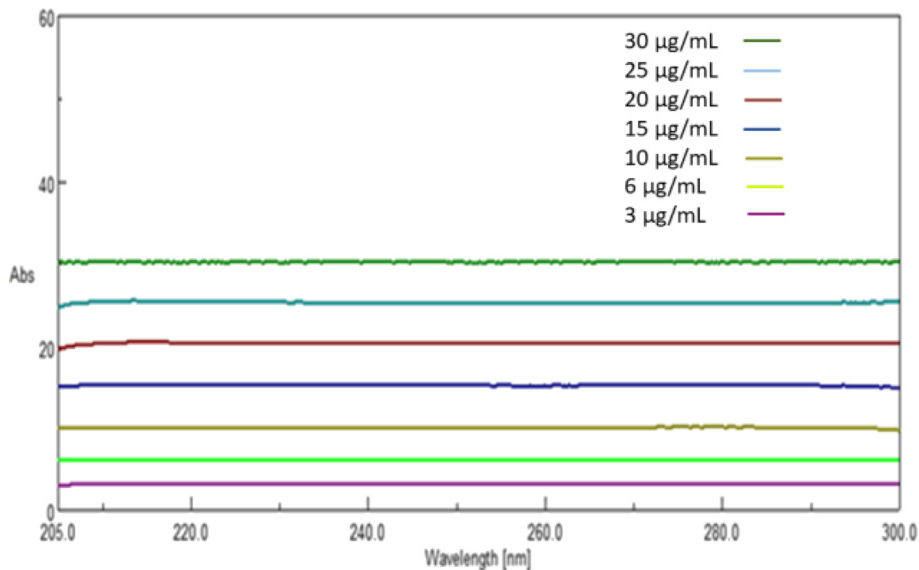
Parameter	PHN				BEN			
	a-centering	AM	AS	CV	a-centering	AM	AS	CV
Wavelength (nm)	244.0	266.1	266.1	274.5	239.1	266.1	266.1	314.1
Intercept	-0.0056	0.0098	-0.0029	-	0.0031	0.0098	-0.0029	-
Slope	0.0561	1.0032	0.0544	-	0.1273	1.0032	0.0544	-
Range (µg/mL)	3–30	3–30	3–30	3–30	3–15	3–30	3–30	3–30
Correlation coefficient	0.9999	0.9999	0.9999	-	0.9999	0.9999	0.9999	-
Trueness[a,b]	99.03 ± 0.52	100.47 ± 0.55	99.04 ± 0.47	99.89 ± 1.05	99.75 ± 1.50	100.34 ± 0.23	99.89 ± 0.82	100.23 ± 0.44
Intra-day precision[a,c]	0.49	0.69	1.26	0.74	0.49	0.61	0.52	0.10
Interday precision[a,c]	0.95	0.45	0.34	0.47	0.39	0.56	0.13	0.28

[a] Average of three experiments.
[b] Mean of the analytes (5.0, 15.0, 25.0 µg/mL) ± standard deviation.
[c] Average of three concentration of the analytes (6.0, 12.0, 24.0 µg/mL).

Table 2

Determination of laboratory prepared mixtures and otic dosage form by the proposed approaches.

PHN:BEN (µg/mL)	PHN					BEN				
	Recovery%[a]									
	a-centering		AM	AS	CV	a-centering		AM	AS	CV
	NS'^	FS'^				NS'^	FS'^			
5 : 10	100.22	99.60	98.24	98.64	100.18	99.51	100.72	100.51	99.54	100.62
10 : 10	99.21	99.31	99.73	101.12	99.22	99.21	100.31	100.25	99.51	100.25
10 : 5	100.11	99.72	101.82	101.81	101.53	100.11	99.83	99.04	99.62	98.76
20 : 5	100.75	100.35	101.15	101.55	101.11	100.75	98.74	98.22	100.66	98.36
13.5: 3.5[b]	98.61	98.74	99.48	98.74	99.63	98.61	99.11	100.85	101.21	100.57
Mean[c]±SD	99.78	99.54	100.12	100.27	100.46	99.22	99.74	99.78	100.11	99.71
	±0.86	±0.59	±1.36	±1.47	±0.99	±1.55	±0.82	±1.11	±0.78	±1.07
R%±SD	99.51	99.61	100.12	100.18	100.45	100.83	100.93	99.04	99.34	100.238
DF#	±0.56	±0.62	±0.38	±0.46	±0.61	±0.59	±0.552	±0.99	±1.06	±0.964
R%±SD	99.66	99.34	100.33	100.57	99.89	99.64	99.79	99.46	100.54	99.64
SA#	±0.32	±0.89	±0.45	±0.311	±0.46	±1.48	±0.64	±0.87	±0.36	±0.88

^**NS'**: results attained using normalized spectrum, **FS'**: results attained using factorized spectrum.
#DF: recovery of dosage form, SA: recovery of standard additions.
[a] Average of three determinations.
[b] Ratio present in Tympanil®.
[c] The mean and SD correspond to the mean and standard deviation of the percentage recovery for all laboratory mixes.

Table 3

Figures of Merit for the calibrations of the (a-centering), (AS), and (AM) methods.

Figures of Merit for Methods	PHN			BEN		
	a-centering	AM	AS	a-centering	AM	AS
LOD (µg/ml)	0.23	0.13	0.24	0.10	0.13	0.24
LOQ (µg/ml)	0.71	0.39	0.73	0.31	0.39	0.73
Sensitivity (S)	0.06	0.10	0.05	0.13	0.10	0.05
Analytical Sensitivity (γ, ml/µg)	14.16	25.32	13.73	32.13	25.32	13.73
MCD (γ-1, µg/ml)	0.07	0.04	0.07	0.03	0.04	0.07

2. Experimental

2.1. Equipment and software

JASCO V-650 double beam UV-VIS spectrophotometer, Quartz cells 1cm were used in measurement.

2.2. Chemicals and reagents

Benzocaine and Phenazone were supplied by SHAHBAA Pharmaceutical Company. the purity percentage was determined by official methods [43] and found to be (99.95 ± 0.65) and (99.65 ± 0.42) for PHN

Table 4

Statistical comparison of the results obtained by the proposed methods and the reference derivative method [26] for the determination of the analytes in bulk powder.

PHN					
Method	a-centering	AM	AS	CV	Reported[b]
[a]Mean	100.33	100.39	99.60	100.16	100.06
SD	0.89	0.51	0.76	0.79	0.88
Variance	0.81	0.26	0.58	0.62	0.78
N	6	6	6	6	6
[c]Student's t test	0.54	0.80	0.95	0.21	-
[d]F test	1.03	3.02	1.35	1.26	-

BEN					
Method	a-centering	AM	AS	CV	Reported[b]
[a]Mean	100.41	100.47	100.35	100.56	100.25
SD	1.22	0.41	0.74	0.46	0.89
Variance	1.48	0.17	0.54	0.21	0.79
N	6	6	6	6	6
[c]Student's t test	0.27	0.57	0.23	0.78	-
[d]F test	1.87	4.65	1.46	3.68	-

[a] Average of six experiments.
[b] reported method is derivative ratio spectra [26].
[c] The corresponding tabulated value of Student's t-test equals to 2.23 at p = 0.05.
[d] The corresponding tabulated value of F equals to 5.05 at p = 0.05.

Table 5

Results of one-way ANOVA for Comparison of the proposed and the reported derivative method [26] for determination of analytes in bulk powder.

Source of variation		Degree of freedom	Sum of squares	Mean square	P value[a]	F value[a]	F critical[a]
PHN	Between columns	4	2.65	0.66	0.39	1.08	2.76
	Within columns	25	15.37	0.61			
	Total	29	18.02	-			
BNZ	Between columns	4	0.35	0.09	0.97	0.14	2.76
	Within columns	25	16.01	0.64			
	Total	29	16.36	-			

[a] There was no significance difference among the methods using one-way ANOVA at $p < 0.05$.

and BEN, respectively.

Tympanil® otic drop labeled to contain 540 mg PHN and 140 mg BEN in 10mL glycerin, were produced by SHAHBAA for pharmaceutical industries, (Aleppo, Syria) Batch No:58.

Ethanol of analytical grade was provided from (Panreac, Barcelona, Spain).

2.3. Solutions preparations

Stock solutions of PHN and BEN were prepared separately at a concentration of 10.0 mg/mL of each compound.

Suitable dilution with ethanol was made from former stock solutions to get PHN and BEN working solution at a concentration of 100.0 μg/mL for each.

2.4. Procedure

Samples solutions containing PHN and BEN separately were prepared with concentration range from (3.0 to 15.0 μg/mL) for BEN and from (3.0 to 30.0 μg/mL) for PHN in a-centering method and from (3.0 to 30.0 μg/mL) for PHN and BEN in a-centering, AM and AS methods. Some calculations and regression equation were made for each method as follows:

2.4.1. For a-centering method

- PHN and BEN spectra are showing partial overlapping spectra and crossing at 266.1 nm (λ_{iso}), the a-centering method could be applied after calculating these factors.
- Absorptivity factor of BEN [$A_{266.1nm}/A_{314.1\ nm}$] was computed via dividing absorbance of various concentrations of pure BEN at 266.1nm/293.1nm.

The point 266.1 nm representing the (λ_{iso}) and the point 293.1 nm representing the point in the extended region of BEN which has the absorbance belonging for BEN only.

- Absorptivity inverse at iso point ($1/a_{266.1nm}$) was calculated by using Excel software via measuring the absorbance at 266.1 nm (λ_{iso}) in zero order spectrum for different concentrations of BEN to get its corresponding absorptivity and then calculate its inverse.
- Normalized spectrum (NS′) was obtained by summing several spectra with different concentrations and dividing them by the total concentration.
- Factorized spectrum (FS′) was attained by dividing the absorption spectrum of BEN by its absorbance value estimated at the (λ_{iso}) 266.1 nm.
- Calibration curves were constructed between absorbance and concentrations for PHN and BEN at their (λ_{Max}) 244.0 nm and 293.1nm, respectively and the regression equations were obtained.

2.4.2. For absorbance subtraction method (AS)

Calibration curve was constructed between absorbance and concentrations for both PHN and BEN at λ_{iso} 266.1nm and the regression equation was attained.

2.4.3. For amplitude modulation method (AM)

Calibration curve was constructed between absorbance and amplitudes for BEN at λ_{iso} 266.1nm after dividing its zero-order spectrum by the normalized spectrum (NS′ $_{BEN}$) then, the regression equation was obtained.

2.4.4. Concentration value method (CV)

D_0 spectrum of the more extended compound BEN was divided by the (NS′ $_{BEN}$), likewise the D_0 spectrum of the less extended compound PHN was divided by the (NS′ $_{PHN}$) after recovering it first by a-centering technique.

2.5. Determination of laboratory prepared combinations and pharmaceutical formulations

Five mixtures including varied proportions of the cited drugs were prepared into a set of 10 mL volumetric flasks. For Tympanil® otic drop a working solution labeled as containing 13.5 μg/mL of [PHN] and 3.5 μg/mL of [BEN] was prepared and five replicates were acquired.

3. Results and discussion

This investigation confirms the quantification of BEN and PHN by the aforementioned spectral techniques. Zero order spectra of the compounds are presented in Fig. 2, where BEN spectrum has extended area over PHN spectrum in the range (314.1–225.0 nm) and intersects with PHN spectrum at three iso-absorptive points: (216.1nm, 225.7nm and 266.1nm), at these three points the two components have equal absorptivity coefficient, however the calculation was performed at λiso 266.1nm since it owns the highest absorbance which improves the results in terms of Trueness and sensitivity Fig. 3.

3.1. Absorptivity Centering method (a-centering)

In order to determine PHN and BEN in binary mixture via (a-centering) method, four spectral factors should be obtained. These factors are: (NS′ $_{BEN}$) the normalized spectrum of BEN, (FS′ $_{BEN}$) the factorized spectrum of BEN, absorptivity factor [a266.1 nm/a314.1nm] of BEN which equals to 1.35 value, and absorptivity inverse factor at λiso (1/aλ266.1) which equals to 18.48 value. Then the following steps are followed after recording the spectrum of any binary mixture of PHN and BEN:

- Multiplying the absorbance of BEN in the mixture at 314.1nm by absorptivity factor of BEN (1.35) to gain its corresponding absorbance A 266.1 nm at (λiso).
- Recovering the Do spectrum of BEN from the mixture by multiplying the previously obtained absorbance A 266.1nm by the computed absorptivity inverse (18.48) then the attained outcome is multiplied by the normalized spectrum (NS′ $_{BEN}$).
- Also, the Do spectrum of BEN could be recovered directly from the mixture by multiplying the obtained A266.1 by factorized spectrum (FS′ BEN).
- Subtracting the obtained D0 spectrum of BEN from the mixture spectrum to recover Do spectrum of PHN.

Table 6
Comparative study between the previous published UV study and the presented study.

Method	Advantages	Drawbacks
Derivative ratio spectra (**DRS**)	✓ Enhanced overlapped spectra resolution. ✓ No requirement for iso point.	• Increased signal to noise ratio. • The need for the best divisor selection test. • Requires choosing an appropriate wavelength increment for derivative. • Requires standard solutions of the interfering compound to be used as a divisor.
Ratio deference (**RD**)	✓ No requirement for isopoint presenting. ✓ No requirement for derivative step.	• Requires the selection of the best divisor. • Requires Standard solutions of the interfering compound to be used as a divisor.
Dual wavelength (**DW**)	✓ No requirement for the presenting of the isopoint.	• Many trails needed to find the two wavelengths with equal absorbance of the interfering compounds.
Derivative (**D_1, D_2, D….**)	✓ Enhanced overlapped spectra resolution. ✓ Increased the sensitivity of the method.	• Increased signal to noise ratio. • Requires choosing an appropriate wavelength increment for derivative. • The need for the zero-crossing point selection.
Q-absorbance ratio (**QR**)	✓ The concentration of each compound can be determined via applying their respective mathematical equation.	• Choosing the ideal wavelengths for measurements • Calculating four factors corresponding to their absorptivity values.
Absorptivity-centering (**a-centering**)	✓ Determines the compounds concentration by applying their zero-order spectra equation at their maxima which enhances trueness and sensitivity. ✓ Getting the zero order spectra of each compound which represents its identity. ✓ Could be applied with partial or sever overlapped spectra.	• Calculating two factors • The existence of normalized or factorized spectra. • Multistep technique.
Amplitude modulation (**AM**)	✓ No requirement for the best divisor concentration selection step. ✓ Only one regression equation is used to determine the concentration of both components. ✓ High sensitivity.	• The existence of normalized spectra stored in the computer.
Absorbance subtraction (**AS**)	✓ No need for complex calculations. ✓ Determines the concentrations of both components using only one regression equation.	• Calculating the absorption factor.
Concentration value (**CV**)	✓ The concentrations of both drugs are determined directly from plateau region. ✓ No requirement for a regression equation. ✓ No requirement for isopoint presenting.	• Requires a complementary spectrophotometric method • The existence of normalized spectra stored in the computer

According to aforementioned points, the D0 spectrum of each PHN and BEN were attained, therefore the concentration of the two compounds were acquired via their regression equations at their corresponding λMax. As a result, obtaining the zero order spectra by this method acts as a fingerprint spectrum which represent the main advantage of this method and allowed testing the purity of both components [32]. On the other hand, this method is considered as a multi-step technique.

3.2. Absorbance subtraction method (AS)

The absorbance of BEN at (λiso 266.1 nm) was computed by utilizing absorptivity factor of BEN which is previously calculated in a-centering method, after that the attained absorbance was subtracted from the absorbance of the mixture at (λiso 266.1 nm) to get the absorbance of PHN. The concentration of PHN and BEN was calculated using the absorbance value gained at (λiso 266.1 nm) via their regression equation constructed at (λiso 266.1 nm).

This approach is easy to apply, does not need any convoluted arithmetic calculations and permits to determine the concentration of the two compounds via only one regression equation at λ_{iso}. The only limitation of this technique is rising mistakes in computing the absorption factor of small concentrations [35].

3.3. Amplitude modulation method (AM)

This approach was applied via dividing the D0 spectra of PHN+BEN binary mixtures by the (NS'$_{BEN}$) in order to get the ratio spectra of PHN+BEN as seen in Fig. 4. PHN concentration could be calculated through substituting the measured amplitude at the extended area of ratio spectrum (314.1–325.0 nm) in the regression equation acquired at (λiso 266.1 nm).

On the other hand, BEN concentration was calculated via subtracting the aforementioned gained constant from amplitude of the division spectra at (λ_{iso} 266.1nm) which represents the total concentration BEN+PHN, then the attained amplitude value was substituted in the regression equation attained at λiso.

This approach does not need any optimization study to obtain the best divisor concentration because it depends on the normalized spectrum as a divisor. Also, the concentration of the two compounds can be estimated via one regression equation at λ_{iso}, otherwise the utilizing of this approach needs the presence of (NS'$_{BEN}$) stored in the computer.

3.4. Concentration value method (CV)

The binary mixture contains BEN and PHN was suggested to apply the concentration value method, BEN spectrum is more extended than PHN. Thus, when the mixture spectrum is divided by (NS'$_{BEN}$), a constant value at plateau area is obtained, which represents the concentration of BEN in the extended part (314.1 nm–325.0 nm) as shown in Fig. 5. PHN concentration is determined by recovering its D_0 spectrum first from mixture by a-centering method and second by dividing the gained D_0 spectrum via (NS'$_{PHN}$) to get a plateau area (205nm–300nm) which represents its concentration as shown in Fig. 6.

In this method, the concentration of both PHN and BEN is determined directly from plateau area without the need for any regression equation, the limitation of this method is the need for a complementary spectrophotometric method to determine the spectrum of the less extended component.

3.5. Method validity and statistical analysis

All the aforementioned procedures were validated according to the ICH criteria [42] as follows:

3.5.1. Range and linearity

The linearity was evaluated through analyzing seven concentrations covering the range from (3.0 to 15.0 μg/mL) for BEN in a-centering method and from (3.0 to 30.0 μg/mL) for BEN and PHN in other submitted approaches.

3.5.2. Trueness

Three variant concentrations of pure PHN and BEN were analyzed at three levels within their linearity and repeated three times. The percentage recoveries exhibit a good trueness of the suggested approaches as presented in Table 1.

3.5.3. Precision

The precision was assessed by analyzing three concentrations of PHN and BEN individually three times on the same day (intra-day) and on three successive days (inter-days). RSD% were evaluated, and a satisfied result was achieved and displayed in Table 1.

3.5.4. Selectivity

Selectivity was accomplished by satisfied percentage recoveries obtained via testing laboratory prepared combinations consisting of varied proportions of PHN and BEN as shown in Table 2.

3.5.5. System suitability

The UV system suitability was verified through calculating the RSD% of the six replicated samples of the both PHN and BEN, separately the values were less than 2%.

3.5.6. Figures of merit

Figures of merit results are significant numerical factors of the analytical method validation. Developing new analytical techniques requires estimating the corresponding analytical merit numbers to report detection capacities and other significant characteristics [44].

In Table 3 Figures of merit such as detection limit (LOD), quantification limit (LOQ), sensitivity, analytical sensitivity (ɣ), and (MCD) minimum concentration difference (ɣ-1) were obtained from the calibration curves corresponding to the (a-centering), (AS), and (AM) methods. From the results shown in Table 3, (a-centering) method was the most sensitive method for BEN quantification using its zero order spectra equation at the λ_{Max}, while (AM) method is considered a good method with high sensitivity for the determination of PHN in the binary mixture of PHN and BEN.

These were satisfactory accepted, proving the fitness of the techniques. However, figures of merit for (CV) method could not be observed because this technique was considered as a graphical determination technique.

3.5.7. Statistical analysis

The four proposed spectrophotometric methods were statistically compared with standard derivative ratio spectra UV method [26] via *t*-test and F-test. The observed results indicate an insignificant difference between the standard and the proposed methods regarding the trueness and precision at P = 0.05. Table 4.

Additionally, the One-way ANOVA test at P > 0.05 level was done with no significant variation in the mean concentrations found using the four proposed methods and the standard derivative ratio spectra UV method as shown in Table 5.

(PHN: $F_{calc(4,25)} = 1.08 < F_{crit(4,25)} = 2.76$), (BEN: $F_{calc(4,25)} = 0.14 < F_{crit(4,25)} = 2.76$).

3.6. Comparative study among developed and announced spectrophotometric approaches

The proposed approaches have several advantages over the announced ones [25, 26] such as getting the zero order spectra of the components in the mixtures which acts as identity-profiles as in

a-centering method, the need for one regression equation to determine both components as in amplitude modulation and absorbance subtraction methods and determined the two components directly from plateau region without any regression equation as in concentration value method. All previous advantages of the proposed approaches beside less drawbacks comparing to the announced ones as presented in Table 6 give them the priority of application in quality control laboratory.

4. Conclusion

The proposed work permits the simultaneous determination of the binary drug mixture of PHN and BEN in the pure form, as well as in the otic pharmaceutical formulation by four spectrophotometric methods, depending on the presence of the more extended spectrum of one component in the binary combination.

These four methods could obtain the concentration of PHN and BEN directly, or even from their computed regression equation depending on the using of the Jasco-spectromanager software and simple mathematical calculations in Excel software. Furthermore, these methods do not require special software like MATLAB, pre-preparation steps or hazardous organic solvents which make them simple, ecofriendly, nondestructive and economic methods.

These spectrophotometric methods provide a good alternative to chromatographic separation method without the use of mobile phases or other separation devices in routine quality control samples [45].

Declarations

Author contribution statement

Duaa Jamal Al Zakri: Performed the experiments; Wrote the paper.
Reem Hasan Obaydo: Analyzed and interpreted the data.
Amir Alhaj Sakur: Conceived and designed the experiments.

Funding statement

Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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