VALIDATED ECO-FRIENDLY SPECTROPHOTOMETRIC METHODS FOR THE DETERMINATION OF ACEFYLLINE PIPERAZINE AND BROMHEXINE HYDROCHLORIDE IN THE PRESENCE OF DOSAGE FORM ADDITIVES[**]

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Four simple, precise, accurate, and eco-friendly spectrophotometric methods manipulating ratio spectra were used for the determination of acefylline piperazine (AC) and bromhexine hydrochloride (BR) in the presence of two dosage form additives – methylparaben (MP) and propylparaben (PP). The dual wavelength in ratio spectra (DWRS) and derivative of double divisor of ratio spectra (DD-RS) methods were used for the simultaneous determination of AC and BR. The first derivative ratio zero crossing method (^{1}DD) was used for the determination of BR. AC can be determined using sequential spectrophotometry, where the ratio subtraction method was applied to remove the extended spectrum of BR followed by applying the dual wave length method. The linearity of the proposed methods was investigated in the ranges 5.00–60.00 and 4.00–60.00 μg/mL for AC and BR, respectively. The selectivity of the developed methods was investigated by analyzing laboratory-prepared mixtures containing different ratios of the cited drugs and additives in addition to the analysis of their pharmaceutical dosage form. The validity of the proposed methods was further assessed using the standard addition technique. The obtained results were statistically compared with those obtained by the reported method, showing no significant difference with respect to their accuracy and precision at p = 0.05.

Keywords: *acefylline piperazine, bromhexine hydrochloride, dual wavelength in ratio spectra, sequential spectrophotometry, derivative of double divisor of ratio spectra.*

ЭКОЛОГИЧНЫЕ СПЕКТРОФОТОМЕТРИЧЕСКИЕ МЕТОДЫ ОПРЕДЕЛЕНИЯ АЦЕФИЛЛИНА ПИПЕРАЗИНА И БРОМГЕКСИНА ГИДРОХЛОРИДА В ЛЕКАРСТВЕННЫХ ФОРМАХ В ПРИСУТСТВИИ ДОБАВОК

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Простые, точные и экологически чистые спектрофотометрические методы, основанные на манипулировании спектрами отношений потоков, использованы для идентификации ацефиллина пиперазина (AC) и бромгексина гидрохлорида (BR) в лекарственных формах в присутствии двух добавок: метилпарабена (MP) и пропилпарабена (PP). Для одновременного определения AC и BR использованы методы двух длин волн (DWRS) и производных спектров двойного деления (DD-RS), для определения BR — метод пересечения кривой первой производной спектра с координатной осью. AC может быть определен с помощью последовательной спектрофотометрии методом вычитания спектров для удаления спектра BR с последующим применением метода двух длин волн. Линейность предложенных методов исследована в диапазоне концентраций 5.00–60.00 и 4.00–60.00 мкг/мл для AC и BR. Селективность разработанных методов изучена путем анализа лабораторно приготов-

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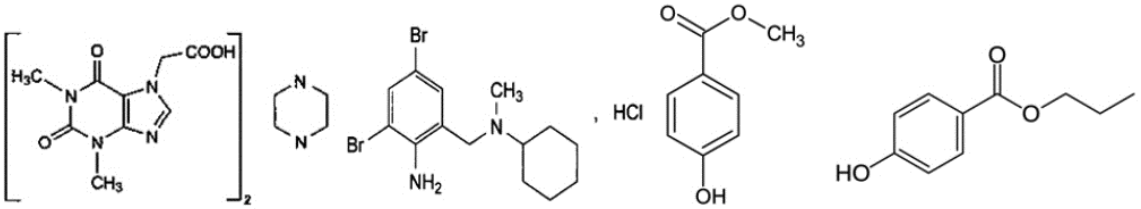
ленных смесей, содержащих разные соотношения указанных препаратов и добавок, в дополнение к анализу их фармацевтической лекарственной формы. Обоснованность методов дополнительно оценена с помощью стандартной методики сложения спектров. Существенных различий в точности полученных результатов с известными данными других авторов при p = 0.05 не обнаружено.

Ключевые слова: *ацефиллин пиперазин, бромгексина гидрохлорид, метод двух длин волн, последовательная спектрофотометрия, метод производных спектров двойного деления.*

Introduction. Acefylline piperazine (AC), 2-(1,3-dimethyl-2,6-dioxopurin-7-yl)acetic acid; piperazine [1] is a stimulant drug of the xanthine chemical class. It is an adenosine receptor antagonist. AC is co-formulated with bromhexine hydrochloride (BR), N-(2-Amino-3,5-dibromobenzyl)-N-methylcyclohexanamine hydrochloride [2], which acts as a mucolytic agent used in the treatment of respiratory disorders associated with viscid or excessive mucus. AC and BR are co-formulated in the dosage form with methylparaben (MP) and propylparaben (PP):



Acefylline piperazine Bromhexine hydrochloride Methyl paraben Propyl paraben

A literature survey reveals several analytical methods reported for the determination of AC alone or in combinations, such as spectrophotometry [3–5] and HPLC [6]. For the determination of BR, different methods were applied, such as spectrophotometry [7, 8], HPLC [9–13], TLC [13–16], and capillary electrophoresis [17].

To the best of our knowledge, only HPLC and the chemometric methods were reported for resolving the mixture under study [18]. Therefore, our main objective is to develop simple, accurate, inexpensive, and eco-friendly spectrophotometric methods for the determination of AC and BR in bulk powder or in the presence of MP and PP in a pharmaceutical dosage without the need for special software (Matlab®), sophisticated instruments, or expensive solvents. The proposed spectrophotometric methods are fast and economical in comparison with the more time-consuming HPLC method, so they can be used as an excellent alternative to HPLC for the determination of the constituents of the cited mixture.

Experimental. *Materials and reagents. Pure Samples.* Acefylline piperazine was kindly supplied by NEHTA Enterprise, India; its purity was found to be 99.90±1.022% according to the reported HPLC method [18]. Bromhexine hydrochloride was kindly supplied by Ven Petrochem &Pharma, India, with a purity of 99.88±0.724% according to the reported HPLC method [18].

Methylparaben and propylparaben were kindly supplied by Sigma Tech Company, Cairo, Egypt, with a purity of 99% for each.

Pharmaceutical formulation. Mucophylline® syrup: Each 5 mL is labeled to contain 100.0 mg of acefylline piperazine, 4.0 mg of bromhexine hydrochloride, 4.5 mg of methylparaben, and 0.5 mg of propylparaben (batch No. 459036 manufactured by MISR Company for pharmaceutical industries, Abu Zaabal, Cairo, Egypt).

Reagents. (a) Methanol. Spectroscopic grade, 99.0% purity (E. Merck, Darmstadt, FRG). (b) Distilled water (Otsuka, Cairo, Egypt).

Standard stock and working solutions. The standard stock solution of AC, BR, MP, and PP was 1.0 mg/mL in 70% methanol, and the working solutions were 0.1 mg/mL in 70% methanol.

Instruments. A Shimadzu dual beam spectrophotometer (Kyoto, Japan), model 1650 UVPC, was used, with 1.00 cm quartz cells. Scans were carried out in the range 200–400 nm with 0.1 nm intervals. The bundled software, UV Probe version 2.21 (SHIMADZU), was used.

Procedures. Spectral characteristics of AC, BR, MP, and PP. The zero-order absorption spectra (^0D) of AC, BR, MP, and PP (60.0, 6.0, 3.0, and 0.5 μg/mL, respectively) were recorded against distilled water as a blank over the range 200–400 nm.

Construction of calibration curves. Aliquots equivalent to 50.0–600.0 μg AC and 40.0–600.0 μg BR were accurately transferred from their corresponding working standard solutions (0.1 mg/mL) into two sepa-

rate series of 10-mL volumetric flasks. Then the volume was completed with distilled water. The spectra of the prepared standard solutions were scanned from 200 to 400 nm.

Dual wavelength in ratio spectra (DWRS). The zero-order spectra of both AC and BR were divided by the standard spectrum of 3 μg/mL of MP. The amplitudes of the obtained ratio spectra were measured at 268.5, 274.2, 243.8, and 253.8 nm. The difference in the amplitudes at the chosen wavelength couple $\Delta P_{268.5\text{-}274.2}$ and $\Delta P_{243.8\text{-}253.8}$ were plotted against the corresponding concentrations of AC and BR, respectively. The regression equations and correlation coefficients were calculated.

Ratio subtraction coupled with the dual wavelength method (RS-DW) (Sequential spectrophotometry) for the determination of AC. The amplitude difference of the absorption spectra of AC at 242.0 and 268.6 nm $\Delta P_{242.0\text{-}268.6}$ was plotted against the corresponding concentration of AC, and the regression equation was calculated.

The first derivative ratio spectra (^{1}DD) method for the determination of BR. For the determination of BR, the absorption spectra of BR were divided by the standard spectrum of 3 μg/mL MP; then the first derivative of the ratio spectra (^{1}DD) with $\Delta\lambda = 4$ nm and the scaling factor 10 were obtained. The amplitude of the first derivative peak was measured at 248.2 nm, where the first derivative of the ratio spectra of AC shows zero crossing. A calibration curve was constructed using the peak amplitude at 248.2 nm versus the corresponding concentrations of BR.

The first derivative of double divisor ratio spectra (^{1}DD-RS) method. For the determination of AC, the stored spectra of AC were divided by the spectrum of the standard mixture solution of BR and MP (5.0 μg/mL of each); then the first derivative of the ratio spectra were obtained at $\Delta\lambda = 4$ nm and the scaling factor 10. The values of the peak amplitude of the obtained derivative ratio spectra were recorded at 295.2 nm, and plotted against the corresponding concentrations of AC. The regression equation and the correlation coefficient were computed.

For the determination of BR, the stored spectra of BR were divided by the spectrum of the standard mixture solution of AC and MP (5.0 μg/mL of each); then the first derivative of the ratio spectra were obtained at $\Delta\lambda = 4$ nm and the scaling factor 10. The values of the peak amplitude of the obtained derivative ratio spectra were recorded at 247.6 nm and plotted against the corresponding concentrations of BR. The regression equation and the correlation coefficient were computed.

Analysis of the laboratory-prepared mixtures by the proposed spectrophotometric methods. Into a series of 10 ml volumetric flasks, accurate aliquots of AC, BR, MP, and PP were transferred from their working solutions to prepare mixtures containing different ratios of the cited drugs and additives. The volumes were completed with distilled water. The spectra of these mixtures were scanned from 200 to 400 nm and stored in the computer. The same procedures during the construction of the calibration curves were applied, and the concentrations of AC and BR were calculated from the corresponding regression equations.

For the determination of AC in laboratory-prepared mixtures using the (RS-DW) method, the laboratory-prepared mixtures were divided by the spectrum of 40.0 μg/mL BR, and then the constant was subtracted from the ratio spectra followed by multiplication with the same divisor spectrum. After that, the amplitude differences of the obtained spectra ($\Delta P_{242.0\text{-}268.6}$) were recorded, and the concentration of AC was obtained from the corresponding regression equation.

Analysis of AC and BR in Mucophylline®syrup by the proposed spectrophotometric methods. Two and half milliliters were accurately transferred from the pharmaceutical dosage form to a 100 mL volumetric flask and diluted to the mark with 70% methanol (solution A). For the AC determination, 0.4mL of the prepared solution A was transferred into a 10 mL volumetric flask, and the volume was completed to the mark with distilled water.

Meanwhile, for the BR determination, 1.2 mL of the prepared solution A was transferred into a 10 mL volumetric flask, and the volume was completed to the mark with distilled water after being spiked with 40 μg of BR from its working standard solution.

The procedures during the construction of calibration curve were preceded; the concentration of each drug was calculated using the corresponding regression equation. The claimed concentration of BR in the preparation was calculated after subtraction of the added concentration (spiked concentration of 4 μg/mL BR analyzed using the same procedure). When carrying out the standard addition technique, different known concentrations of the pure standard of each drug were added to the pharmaceutical dosage form before proceeding in the previously mentioned methods.

Results and discussion. Spectrophotometry is a feasible solution for developing a simple, easy, and selective method compared to the high cost of HPLC. But the main obstacle facing spectrophotometry is the overlapping spectra of co-formulated drugs and additives. The cited mixture (AC, BR, MP, and PP) exhibits significant interference, as shown in Fig. 1, which hinders resolving the studied combination by direct and classical derivative spectrophotometric methods. Hence, their simultaneous determination necessitates a mathematical treatment of the absorption data in order to omit the interference imposed by each drug and additives while determining the other, or merging some traditional analytical techniques together to overcome the spectral interference.

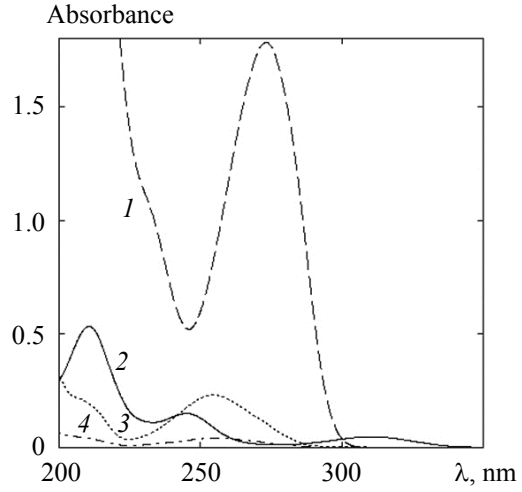


Fig. 1. The zero-order absorption spectra of 60 μg/mL AC (1), 6 μg/mL BR (2), 3 μg/mL MP (3), and 0.5 μg/mL PP (4) using distilled water as a blank.

Our main objective is to establish simple spectrophotometric methods for the determination of AC and BR in the presence of the pharmaceutical dosage form additives without prior separation.

The biggest challenge in the determination of the cited drugs is to determine the minor component BR. Subsequently, there is a need to increase its concentration in the dosage form solution using the standard addition technique to eliminate the deviation from Beer's law, which occurs when the transmittance values approach 0 or 100% [19]. This is done by adding fixed amounts of the standard BR to the dosage form solution to each experiment and then subtracting its concentration before calculating the claimed concentration of BR.

In addition, Fig. 1 shows that methyl and propyl parabens exhibit almost the same spectrum; therefore, they could be considered as a single component. PP represents 10% of the total additive concentrations, so we can consider the combination as a ternary mixture.

Dual wavelength in ratio spectra (DWRS). Saad [20] merged two of the well-established spectrophotometric methods, dual wavelength [21, 22] and the ratio difference [23, 24], together, which gave rise to this simple smart dual wavelength in the ratio spectra spectrophotometric method, which resolves the overlapped spectra of ternary mixtures, where each component can be selectively determined after removing the interference of the other components.

In this method one single division step was used for the determination of both AC and BR in their mixtures. The scanned spectra were divided by the spectrum of 3 μg/mL of MP. The obtained ratio spectra in Fig. 2 shows MP and PP as straight lines with a constant amplitude, where BR shows equal amplitudes at 268.5 and 274.2 nm, while AC shows equal amplitudes at 243.8 and 253.8 nm. Therefore, the difference in amplitudes at these two wavelengths $\Delta P_{268.5\text{-}274.2}$ and $\Delta P_{243.8\text{-}253.8}$ selectively corresponds to the AC and BR concentrations.

No significant effect was observed upon trying MP or PP as a divisor, but MP was used as it exhibits a higher absorptivity and represents 90% of the total additives in the dosage form. Different concentrations of MP were tried, but the best results were obtained upon using 3μg/mL MP as a divisor for the determination of both AC and BR, regarding the maximum selectivity and minimal noise.

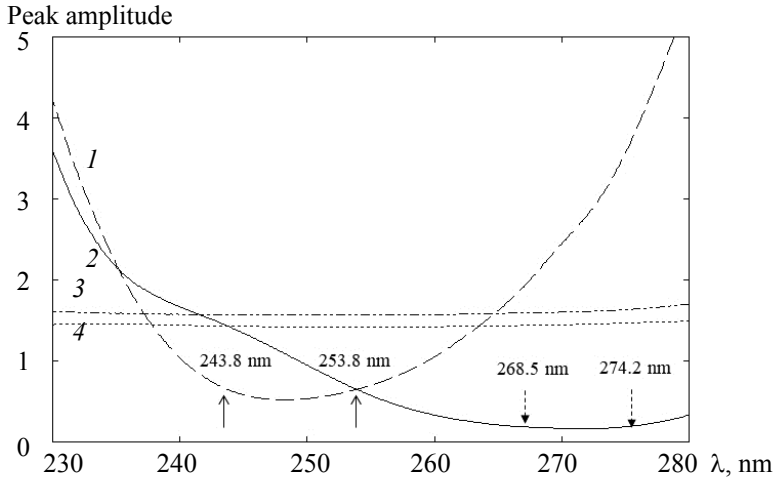


Fig. 2. Ratio spectra of 10 μg/mL AC (1), 10 μg/mL BR (2), 5 μg/mL MP (3), 5 μg/mL PP(4) using 3 μg/mL MP as a divisor and distilled water as a blank.

The ratio subtraction coupled with dual wavelength method (RS-DW) for the AC determination (sequential spectrophotometric method). This method depends on the presences of an extended spectrum in a ternary mixture [25], where ratio subtraction method [26] is applied to remove it. Then any spectrophotometric method can be applied to resolve the obtained binary mixture [22–24, 27, 28]. For the identification of substances in ternary mixtures the following theory can be used [25, 29, 30]. Let the mixture be composed of substances $X + Y$, where X = AC + MP + PP and Y = BR. The zero-order absorption spectra of the laboratory-prepared mixture (AC, BR, MP, and PP) divided by the spectrum of the standard solution of 40.00 μg/mL BR (Y' = divisor) produced the ratio spectra (X/Y' + constant), as is shown in Fig. 3a. The constant was subtracted from the ratio spectra, as shown in Fig. 3b, followed by the multiplication of the obtained spectra with the divisor (Y'), as shown in the Fig. 3c.

Finally, the original spectra (X) were obtained in Fig. 3c. Then, AC can be determined by applying the dual wavelength method. Two wavelengths were selected (242.0 and 268.6 nm), where MP and PP showed the same amplitudes, while AC showed a significant difference in these two amplitude values at these two selected wavelengths (Fig. 4). Different concentrations of the divisors were tried, but the best results regarding selectivity were obtained using 40.00 μg/mL BR as the divisor.

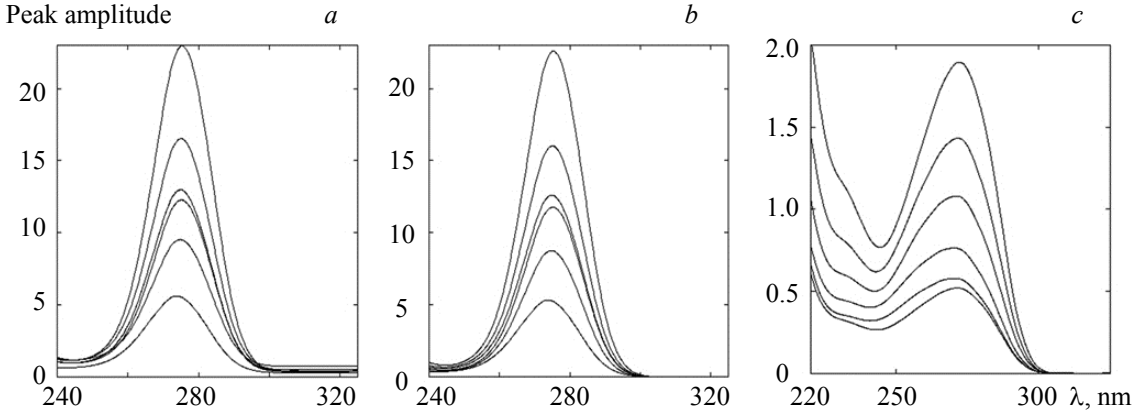


Fig. 3. a) Ratio spectra of laboratory-prepared mixtures of AC, BR, MP, and PP using 40 μg/mL BR as a divisor and distilled water as a blank; b) the obtained ratio spectra after the subtraction of the constant; c) the obtained spectra of the mixture AC, MP, and PP after multiplication by the divisor 40 μg/mL BR.

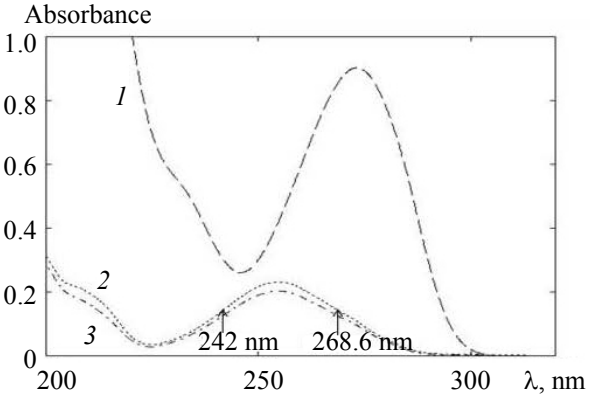


Fig. 4. The zero-order absorption spectra of 30 μg/mL AC (1), 3 μg/mL of both MP (2), and PP (3) using distilled water as a blank.

The first derivative ratio spectra method (^{1}DD) for BR determination. This method was investigated to determine BR but failed in the determination of AC even when different divisors (BR or MP) were tried. For the determination of BR the 3 μg/mL MP spectrum was used as a divisor and gave good selectivity. The first derivative of the ratio spectra (^{1}DD) was found to be convenient for the determination of BR at 248.2 nm, where the spectrum of AC showed zero crossing, as shown in Fig. 5. Different Δλ values were tried (2–8), where Δλ = 4 showed a suitable signal-to-noise ratio and the spectra showed a good resolution. Different scaling factor values were tried (1–100), and scaling factor 10 was found to be suitable to enlarge the signal of both compounds. A good linearity was obtained between the peak amplitude of ^{1}DD at 248.2 nm and the corresponding concentrations.

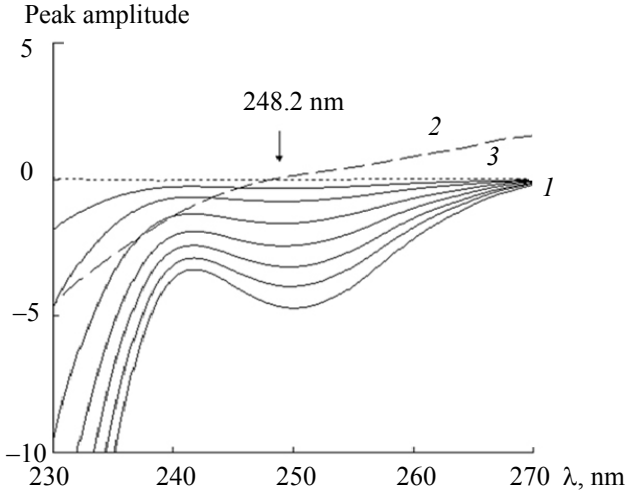


Fig. 5. First derivative of ratio spectra of 4–60 μg/mL BR (1), 10 μg/mL AC (2), and 5 μg/mL PP (3) using 3 μg /mL MP as a divisor and distilled water as a blank.

The derivative of double divisor of the ratio spectra method (DD-RS). Dinc and Onur [31] developed the derivative of double divisor of ratio spectra method for resolving ternary mixtures without previous separation. DD-RS is based on dividing the ternary mixture by the spectrum of the standard mixture of two of the three compounds in the title mixture as a double divisor (DD); then the first derivative of this ratio spectrum is obtained [30–33]. DD-RS permits the determination of AC in its mixture by measuring the peak amplitude of the first derivative double divisor spectra at 295.2 nm, as shown in Fig. 6a. The spectrum of the laboratory-prepared mixture containing BR and MP (5 μg/mL of each) was used as a double divisor. Meanwhile, BR was determined at 247.6 nm, as shown in Fig. 6b, using the spectrum of the laboratory-prepared mixture

containing AC and MP (5 μg/mL of each) as a double divisor. Different Δλ values were tried, in which Δλ = 4 showed a suitable signal-to-noise ratio and the spectra showed a good resolution. Moreover, different scaling factor values were tried, and scaling factor 10 was found to be suitable. To select the working wavelength, the first derivative of the ratio spectra of the pure drugs and their ternary mixtures (after division by the double divisor) should be obtained by overlapping the two derivative spectra. The coincident points of choice were 295.2 and 247.6 nm for the determination of AC and BR, respectively (Fig. 6). A good linearity was obtained between the peak amplitude of the first derivative double divisor spectra at selected wavelengths and the corresponding concentrations of the studied drugs.

The corresponding concentration ranges and calibration equations for the proposed methods are listed in Table 1.

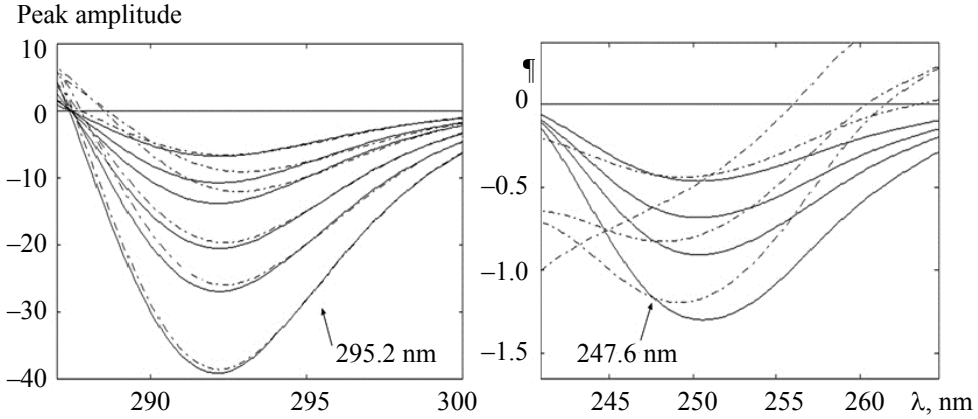


Fig. 6. The coincident spectra of the first derivative of the ratio spectra of: a) pure AC (solid line) and laboratory prepared mixture of AC, BR, MP and PP (dased line) using the spectrum of the laboratory-prepared mixture containing 5.00 μg/mL of BR and MP as a double divisor. b) pure BR (solid line) and laboratory-prepared mixture (dased line) using the spectrum of the laboratory-prepared mixture containing 5.00 μg/mL of AC and MP as a double divisor.

TABLE 1. Assay Validation Sheet of the Proposed Spectrophotometric Methods for the Simultaneous Determination of AC and BR

Parameter	AC			BR		
	[DWRS] ($\Delta p_{268.5–274.2}$)	[RS-DW] ($\Delta p_{242–268.6}$)	[DD-RS] at λ= 295.2 nm	[DWRS] ($\Delta p_{243.8–253.8}$)	[DR] at λ= 248.2 nm	[DD-RS] at λ= 247.6 nm
Range, μg/mL	5–60	5–60	5–60	4–60	4–60	4–60
Linearity						
Slope	0.1005	0.0179	0.4630	0.0731	0.0752	0.0377
Intercept	0.0693	0.0100	0.3705	0.0756	0.0841	0.0459
Correlation coefficient *r*	0.9998	0.9998	0.9998	1	1	0.9999
Accuracy						
(Mean ± SD)	100.78±0.776	100.76±0.884	99.13±0.624	100.71±0.982	100.78±0.771	100.80±0.708
*Precision (*RSD*)*						
Intraday[a]	0.797	0.944	0.408	0.533	0.562	0.780
Interday[b]	0.868	1.028	0.874	1.006	1.026	0.920

[a]The intraday RSD % (n = 3), average of three concentrations (25, 35, and 45 μg/mL) for both AC and BR repeated three times within the day.

[b] The interday RSD % (n = 3), average of three concentrations (25, 35, and 45 μg/mL) for both AC and BR repeated three times in three successive days.

TABLE 2. Results Obtained for the Determination of AC and BR in the Laboratory-Prepared Mixtures in the Presence of MP and PP by the Proposed Spectrophotometric Methods

Concentration (µg/mL)				AC			BR		
				[a] Recovery %					
AC	BR	MP	PP	[DWRS] ($\Delta p_{268.5–274.2}$)	[RS-DW] ($\Delta p_{242–268.6}$)	[DD-RS] at λ = 295.2 nm	[DWRS] ($\Delta p_{243.8–253.8}$)	[DR] at λ = 248.2 nm	[DD-RS] at λ= 247.6 nm
10	10	2	3	99.49	99.44	99.76	99.10	99.90	99.50
15	20	1	1	100.82	100.56	99.76	100.37	99.14	100.88
20	30	1	3	101.41	101.68	98.47	100.75	99.38	100.42
30	15	3	2	100.16	99.44	99.59	99.99	98.31	98.87
40	20	4	1	100.68	101.68	100.09	98.52	98.30	100.88
60	15	3	1	98.42	99.63	97.55	98.90	101.41	101.34
Mean				100.16	100.41	99.20	99.61	99.41	100.32
SD				1.072	1.071	0.983	0.891	1.162	0.944
RSD%				1.070	1.067	0.991	0.895	1.169	0.941

[a] Average of three determinations.

To assess the selectivity of the proposed spectrophotometric methods, different laboratory-prepared mixtures containing different ratios of the cited drugs and additives were analyzed by the proposed methods. The obtained results for the determination of AC and BR in laboratory-prepared mixtures were satisfactory, as shown in Table 2.

TABLE 3. Results Obtained from the Analysis of Mucophylline® Syrup Using the Proposed Spectrophotometric Methods and Application of Standard Addition Technique

	Method	Found % * ± RSD	Standard Addition		
			Added, µg/mL	Found, µg/mL	Recovery, %*
AC	[DWRS] ($\Delta p_{268.5–274.2}$)	101.62 ±1.020	10	10.05	100.50
			20	20.31	101.55
			30	30.12	100.40
			Mean ±RSD		**100.82±0.632**
	[RS-DW] ($\Delta p_{242–268.6}$)	99.88 ±1.030	10	9.94	99.40
			20	20.34	101.70
			30	30.28	100.93
			Mean ±RSD		**100.68±1.162**
	[DD-RS] at λ= 295.2 nm	100.85 ±0.537	10	9.97	99.70
			20	20.31	101.55
			30	30.32	101.07
			Mean ±RSD		**100.77± 0.953**
BR	[DWRS] ($\Delta p_{243.8–253.8}$)	99.10±1.193	10	9.89	98.90
			20	19.99	99.95
			30	30.41	101.37
			Mean ±RSD		**100.07±1.239**
	[DR] at λ= 248.2nm	101.12 ±1.084	10	10.13	101.30
			20	20.37	101.85
			30	30.28	100.93
			Mean ±RSD		**101.36±0.457**
	[DD-RS] at λ= 247.6 nm	98.18±0.860	10	10.05	100.50
			20	20.42	102.10
			30	30.59	101.97
			Mean ±RSD		**101.52±0.906**

*Average of three determination

AC claimed to be 20 µg/ml, and BR: 2.4 µg/ml (after subtraction of spiked BR concentration equivalent to 4 µg/ml).

The proposed methods were applied for the determination of AC and BR in Mucophylline® syrup, and the validity of the proposed method was further assessed by applying the standard addition technique, showing good results, as presented in Table 3.

Method validation. The proposed spectrophotometric methods were validated in compliance with the ICH guidelines [34], as shown in Table 1. The data showed that the methods were accurate, precise, and specific over the specified range.

Statistical analysis. Statistical comparison of the results obtained by the proposed methods and the reported HPLC method is shown in Table 4. The calculated t and F values were less than the theoretical ones. This indicated that there was no significant difference between the proposed and reported methods [18] with respect to their accuracy and precision.

TABLE 4. Statistical Comparison between the Results Obtained by the Proposed Spectrophotometric Methods and Those Obtained by the Reported Method for the Analysis of the Pure form of AC and BR

AC				
Parameter	DWRS	RS-DW	DD-RS	Reported Method [b]
Mean	100.78	100.76	99.13	99.90
SD	0.776	0.884	0.619	1.021
n	5	5	5	5
Variance	0.602	0.781	0.383	1.042
t-test (2.306) *	1.536	1.425	1.443	
F (6.39) *	1.73	1.33	2.72	
BR				
Parameter	DWRS	DR	DD-RS	Reported Method [b]
Mean	100.71	100.78	100.8	99.88
SD	0.982	0.771	0.714	0.723
n	5	5	5	5
Variance	0.964	0.594	0.510	0.523
t-test (2.306) [a]	1.522	1.905	2.026	
F (6.39) [a]	1.84	1.14	1.03	

[a]These values represent the corresponding tabulated values of *t* and *F* at $p = 0.05$.
[b] Reported HPLC method using C18 column (5µm, 25 cm×4.6 mm I.D.), 0.05 M potassium dihydrogen phosphate (pH 3):acetonitrile (50:50, v/v) as the mobile phase at a flow rate of 1.5 mL/min and UV detection at 245 nm.

Conclusion. The proposed spectrophotometric methods are simple, sensitive, selective, and eco-friendly; therefore, they can be applied in the routine analysis of AC and BR in their pure form and pharmaceutical formulation. The suggested spectrophotometric methods are simpler than the reported chromatographic and chemometric ones as they require no sample preparation, buffer preparation, expensive solvents, sophisticated liquid chromatographic instruments, and special software or data preprocessing.

Just one division step was able to determine both AC and BR simultaneously in the dual wavelength in the ratio spectra method. In the sequential spectrophotometric method, the derivative step was not applied and the signal-to-noise ratio was enhanced contrariwise the DD and DD-RS. But its main drawback was that several steps were applied to determine AC in laboratory-prepared mixtures. Searching for the coincident point which will be the wavelength of interest is the main challenge in DD-RS.

Conflict of interest. The authors declare no conflict of interest.

REFERENCES

1. *Clarke's Analysis of Drugs and Poisons*, 4th ed., Pharmaceutical Press, London (2011).
2. *The British Pharmacopoeia*, Her Majesty's Stationary Office, London (2013).
3. A. El-Gindy, S. Emara, G. M. Hadad, *Farmaco*, **58**, 377–390 (2003).
4. M. Korany, A. Wahbi, S. Mandour, M. Elsayed, *Anal. Lett.*, **18**, 21–34 (1985).
5. N. A. Zakhari, M. I. Walash, S. M. Ahmed, *Farmaco*, **46**, 601–613 (1991).
6. G. Ragab, H. Saleh, M. El-Henawee, O. F. Elsayed, *Eurasian J. Anal. Chem.*, **11**, 197–210 (2016).

7. I. H. Habib, M. E. Hassouna, G. A. Zaki, *Farmaco*, **60**, 249–254 (2005).
8. D. M. Vishal, G. V. Tukaram, J. P. Pramod, *Int. Res. J. Pharm.*, **3**, 197–201 (2011).
9. G. Bazylak, L. J. Nagels, *J. Pharm. Biomed. Anal.*, **32**, 887–903 (2003).
10. M. Javanbakht, M. H. Namjumanesh, B. Akbari-Adergani, *Talanta*, **80**, 133–138 (2009).
11. L. Shujuan, L. Zhao, X. Liu, F. Zhou, S. Jiang, *J. Food Drug Anal*., **12**, 306–310 (2004).
12. S. E. Vignaduzzo, T. S. Kaufman, *J. Liq. Chromatogr. Relat. Technol*., **36**, 2829–2843 (2013).
13. A. Tyagi, N. Sharma, K. Mittal, R. Mashru, T. Bhardwaj, J. Malik, A. Thakkar, *Pharm. Anal. Acta*, **6**, 1–9 (2015).
14. H. N. Dave, R. C. Mashru, A. K. Patel, *J. Pharm. Sci. Res*., **2**, 143–148 (2010).
15. M. V. Dhoka, V. T. Gawande, P. P. Joshi, *J. Pharm. Sci. Res*., **2**, 477–483 (2010).
16. E. Sumarlik, G. Indrayanto, *J. Liq. Chromatogr. Relat. Technol*., **27**, 2047–2056 (2009).
17. M. Pospısilova, M. Polasek, V. Jokl, *J. Pharm. Biomed. Anal*., **24**, 421–428 (2001).
18. A. El-Gindy, S. Emara, H. Shaaban, *J. Pharm. Biomed. Anal*., **43**, 973–982 (2007).
19. J. Kenkel, *Analytical Chemistry for Technicians*, 3rd ed., CRC Press, London (2003).
20. A. S. Saad, *Spectrochim. Acta A: Mol. Biomol. Spectrosc*., **147**, 257–261 (2015).
21. S. Shibata, *Angew. Chem. Int. Ed. Engl*., **15**, 673–679 (1976).
22. S. Bindaiya, S. Bankey, D. Jain, *Int. J. Chem. Technol. Res*., **2**, 11–15 (2010).
23. E. S. Elzanfaly, A. S. Saad, A. A. B. Abd-Elaleem, *Saudi Pharm. J*., **20**, 249-253 (2012).
24. H. W. Darwish, S. A. Hassan, M. Y. Salem, B. A. El-Zeany, *Spectrochim. Acta A: Mol. Biomol. Spectrosc*., **104**, 70–76 (2013).
25. H. W. Darwish, S. A. Hassan, M. Y. Salem, B. A. El-Zeany, *Int. J. Spectrosc*., **2013**, 1-8 (2013).
26. M. R. El-Ghobashy, N. F. Abo-Talib. *J. Adv. Res*., **1**, 323–329 (2010).
27. M. G. El-Bardicy, H. M. Lotfy, M. A. El-Sayed, M. F. El-Tarras, *JAOAC Int*., **91**, 299–310 (2008).
28. F. Salinas, J. B. Nevado, A. E. Mansilla, *Talanta*, **37**, 347–351 (1990).
29. H. A. Merey, S. S. El-Mosallamy, N. Y. Hassan, B. A. El-Zeany, *Spectrochim. Acta A: Mol. Biomol. Spectrosc*., **160**, 50–57 (2016).
30. H. A. Merey, N. K. Ramadan, S. S. Diab, A. A. Moustafa, *Spectrochim. Acta A: Mol. Biomol. Spectrosc*., **125**, 138–146 (2014).
31. E. Dinc, F. Onur, *Anal. Chim. Acta*, **359**, 93–106 (1998).
32. E. Dinç, *Talanta*, **48**, 1145–1157 (1999).
33. H. A. Merey, S. S. El-Mosallamy, N. Y. Hassan, B. A. El-Zeany, *Anal. Chem. Lett*., **6**, 70–85 (2016).
34. *ICH, Q2 (R1) Validation of Analytical Procedures, in: Proceeding of the International Conference on Harmonization*, Geneva (2005).