



Simultaneous Quantification of Diaveridine and Sulfadimidine by Derivative and Ratio Derivative UV Spectroscopy



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Abstract

Derivative spectrophotometry for two sulfa drugs (Diaveridine and sulfadimidine) was carried out for their simultaneous quantification in their binary mixtures. The optimum conditions were studied. The zero crossing wavelengths for Diaveridine (DV) in presence of sulfadimidine (SDM) were 235, 273, 242, 258, 244 nm, 238 and 289, 224 nm for 1st, 2nd, 3rd and 4th derivatives, respectively. The linear range for DV was 2.5-35.0 µg/mL and that for SDM was 1.0-25.0 µg/mL. LODs for DV were 2.32, 2.50, 0.76 and 1.89 µg/mL with LOQs 7.73, 8.33, 2.55 and 6.31 µg/mL for 1st, 2nd, 3rd and 4th derivatives, respectively. LODs for SDM were 3.19, 2.59, 3.22 and 2.29 µg/mL with LOQs of 10.62, 8.63, 10.73 and 7.64 µg/mL for 1st, 2nd, 3rd and 4th derivatives, respectively. Moreover, ratio derivative method (1DD) was used for the quantification of both drugs; ranging from 10.0-25.0 µg/mL for both drugs. 1DD values were measured at 223 and 254 nm, for diaveridine and sulfadimidine, respectively. The obtained results were compared statistically by the Paired t-test and the variance ratio F-test.

Keywords: Diaveridine; sulfadimidine; sulfa drugs; derivative spectrophotometry.

1. Introduction

Sulfa drugs among several classes of pharmaceutical-antimicrobial agents are of special concern due to the conceivable formation of resistant bacterial strains [1-3]. In the veterinary field, dihydrofolate reductase inhibitors (DHFRIs) for example, diaveridine (Scheme 1) act as potentiators in a mix with sulfonamides (SFA) such as sulfadimidine (Scheme 1) for the counteractive action of bacterial and protozoal diseases of animals [4, 5]. Sulfonamides are an important class of antibacterial drugs used in medicine and veterinary practice. Sulfa medications are broadly utilized in the treatment of contaminations [5-8], particularly for patients narrow minded to antibiotics. The accomplishment of these restorative specialists has made the study of sulfonamides a significant branch of research and a

significance part in pharmaceutical sciences. Sulfonamides comprehensively used in the treatment of urinary tract contaminations. The official technique of British Pharmacopoeia [9], and United States Pharmacopoeia [10], depicts nitrite titration strategy for the examination of sulfa-drugs. Different techniques chromatography [11, 12], electro analytical methods [13-16] and spectrofluorometric [17] were used. Spectrophotometry is the most important strategy [18, 19], which has already been reported for the drug quantification as a single component [20-28]. On the basis of the overlap in the spectra of two compounds, they cannot be analyzed simultaneously by direct spectrophotometry. Derivative spectrophotometry permits the conceivable outcomes for expanding selectivity of the analytical methods without the need for prior separation and masking agent procedure [29-31]. It comprises of computing and plotting one of the

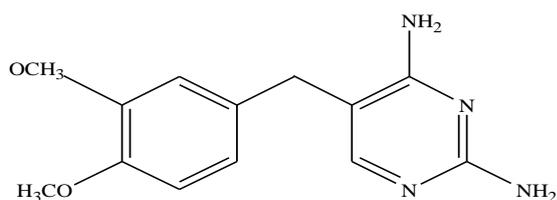
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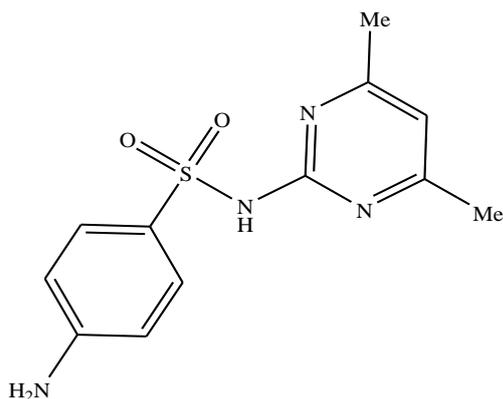
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mathematical derivatives of a spectral curve.



Diaveridine



Sulfadimidine

Scheme 1. Structural formulae of diaveridine and sulfadimidine

The purpose of this work is to quantify sulfadimidine and diaveridine in their mixtures utilizing derivative spectrophotometry. The method can directly determine the cited drugs simply. The developed calibration curves were utilized in deciding the concentration of these drugs in different pharmaceutical formulations available with great accuracy and precision. This method is based on the measurements of the absolute value of the derivative spectrum of the mixture of two compounds at a wavelength value where the absorbance of one component of the mixture goes to zero. At this wavelength, the intensity is directly proportional to the other component.

The applications of derivation enable the separation of hidden signals and use them for quantitative purposes, because derivative leads to sharper zero-order bands and gives higher signals in the resulting spectra. The spectra characteristics, for example peak height and noise level based on the parameters choice [30].

2. Experimental

2.1. Apparatus

Shimadzu 1601 double beam spectrophotometer was used for measuring the absorbance in a cuvette with an optical path length of 1 cm, directed to an IBM-PC computer with a Lexmark printer that was utilized for absorbance signals and data treatment.

2.2. Reagents

All reagents used throughout this study are of analytical grade. Diaveridine was obtained from El Nassr Pharmaceutical Chemicals Company, Egypt. Sulfadimidine was acquired from the Alexandria Company for Pharmaceutical and Chemical Industries, Alexandria, Egypt. Enteroquin® powder for oral suspension subjected in Amoebic and Bacillary Dysenteries and intestinal infections was obtained from the Arab Drug Company, Cairo, Egypt. New-Cox® powerful anticoccidial water soluble powder was purchased from Atco Pharama for Pharmaceutical Industry, Cairo, Egypt. While Sulfadine-K® water soluble powder was the product of United Biomid Pharama for Pharmaceutical Industry, Cairo, Egypt.

2.3. Analysis by derivative UV spectrophotometry

Derivative spectrophotometry method proposed by O'Haver [32] has been applied effectively for the simultaneous quantification of drugs in their mixtures. In this investigation, various orders of derivative and various types of measurements were suggested; i.e. first-, second-, third-, fourth-derivative and first ratio-spectra first derivative for the same purpose.

2.3.1. Derivative spectrophotometric methods (1D, 2D, 3D and 4D) for determination of diaveridine and sulfadimidine

Aliquots of (1 mL) from diaveridine and sulfadimidine standard working solution, equivalent to 100 µg/mL for both drugs, was transferred separately into two 10 mL volumetric flasks, and then the volume was completed with methanol. The spectra of these two solutions were scanned against a blank solution (methanol) and their absorptions were computed. The concentration of each drug was determined by constructing a calibration graph between the concentration of the drug as abscissa and

$dA/d\lambda$ as ordinate at the determined zero crossing point of the other one. The 1D, 2D, 3D and 4D spectra were recorded under certain selected instrumental parameters as, $\Delta\lambda$, scaling factor and wavelength range, after that the working wavelengths of the two drugs, at the zero crossing points were recorded.

2.3.2. Analysis by ratio spectra first order derivative UV spectrophotometry

Salinas et al. [33] introduced the technique of ratio spectra derivative spectroscopy. The decided method is based on the use of the first-derivative of the ratios of the spectra. The stored spectra of the working standard solutions of diaveridine (2.5-35.0 $\mu\text{g/mL}$) were divided several times by the spectrum of working standard solution of sulfadimidine each time with different concentrations (2.5, 5.0, 7.5, 10.0 $\mu\text{g/mL}$) in order to obtain the most suitable concentration used as a divisor in the determination of diaveridine ratio spectra. The first derivative then was applied under certain selected instrumental parameters. The peak amplitudes were quantified, then, their values were plotted against diaveridine related concentrations. The statistical analyses of these calibration graphs were obtained using least squares method. Similarly, the stored spectra of the working standard solutions of sulfadimidine (1.0-25.0 $\mu\text{g/mL}$) were divided several times by the spectrum of working standard solution of diaveridine each time with various concentrations (2.0, 4.0, 6.0, 8.0, 10.0 $\mu\text{g/mL}$) after that, the first derivative was applied. The peak amplitudes were plotted against the corresponding concentrations of sulfadimidine, the statistical analyses of these calibration graphs were obtained using least squares method.

3. Results and discussion

3.1. Optimization of chemical parameters

Non polar solvents were not considered since they do not present good solubility and could become a future source of error. Different solvents were studied to develop suitable methods of analysis such as methanol, ethanol, acetone, acetonitrile, 0.1 mol/L HCl and 0.1 mol/L NaOH, the criteria employed were the sensitivity of the method and availability of the solvent. So that, from solvent effect studies and the spectral behavior of diaveridine and sulfadimidine, methanol was the solvent of choice for all the suggested methods owing to the high solubility of the

drugs in it, and also there is no shift in their absorbance maxima in methanol. Also extraction of the drug with methanol from pharmaceutical preparations eliminates the interference from most common excipients present, under these conditions, the simultaneous determination is more effective and the sensitivity for the determination of the cited drugs is improved. Further, in this solvent both drugs are stable for at least 24 h which is a good feature needed for any applicable method.

3.2. Selection of optimum apparatus conditions

The main instrumental parameter affecting the shape of the derivative spectra is the wavelength increment over which the derivatives are obtained ($\Delta\lambda$). This parameter needs to be optimized to give a well resolved large peak, to give good selectivity and higher sensitivity in the determination. Generally, noise decreases with an increase of $\Delta\lambda$, thus decreasing the fluctuation in a derivative spectrum. However, if the value of $\Delta\lambda$ is too large, the spectral intensity signal of the derivative deteriorates. For diaveridine and sulfadimidine when the $\Delta\lambda$ value was more than 10 nm, the signal amplitudes decrease slightly.

The scale factor must be studied in order to observe whether the system presents a distortion effect of the spectra. Further, the selection of this parameter permits improvement of the reading of the analytical signal. By using the first derivative, the scaling factor was varied as; 2, 4, 8 and 10, a value of 10 was selected because it presents maximum sensitivity without affecting the signal/ noise ratio. By using the second derivative, the scaling factor was varied as; 10, 20, 30 and 100, a value of 100 was selected because it presents maximum sensitivity. While in the third and fourth derivative method, the measurements were done at scaling factor equal 1000.

3.3. Selection of optimum mathematical parameters

Preliminary tests to optimize the method concerned, in particular, the selection of the more convenient working wavelengths. Fig. (1) shows the zero-order UV spectra of DV and SDM. It is quite clear that there is extensive spectral overlap, thus conventional UV spectrophotometry cannot be used for the individual determination of either drug in their binary mixture. The study of their derivative spectra may overcome this difficulty.

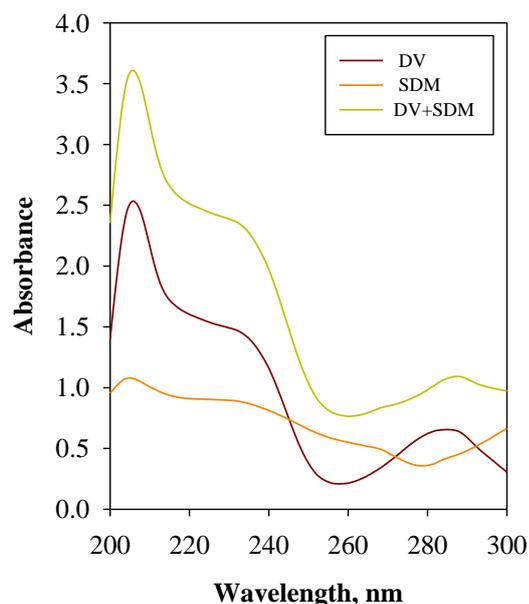


Fig. 1. Zero-order absorption spectra for 15.0 µg/mL DV and 10.0 µg/mL SDM in methanol and their mixture.

3.4. Derivative spectrophotometric methods for simultaneous determination of diaveridine and sulfadimidine

3.4.1. Selection of analytical wavelength

The first order spectra of DV and SDM, also their zero crossing wavelengths were indicated in Fig. 2, a. The selected wavelength, for the determination of DV was 242 nm; at this wavelength the amplitude is proportional to DV concentration only (zero-crossing point for SDM). On the other hand, the selected wavelength for the determination of SDM was 258 nm, because at this wavelength SDM peaks have amplitude values while the corresponding DV peaks read zero (zero-crossing point for DV). So these wavelengths would be examined to be the optimum working wavelengths for simultaneous determination of DV and SDM in their binary mixtures.

The good resolution of the second-derivative spectra allowed selecting the optimal working wavelengths from several zero-crossing wavelengths, those which exhibited the best linear response to

analyte concentration and higher sensitivity. The second-derivative spectra of DV and SDM are shown in Fig. (2, b), also their corresponding zero-crossing wavelengths were indicated in these figures. Among these, $\lambda=235$ nm for determination of DV and $\lambda=273$ nm for determination of SDM were selected as working wavelengths (shown in the figure), in that measurements of the absolute value of the total derivative spectra taken at these wavelengths gave the best response to the analyte concentration.

Figure (2, c) shows the third order (3D) spectra of DV and SDM, and also their corresponding zero-crossing wavelengths were indicated in this figure (244 nm for DV and 238 nm for SDM).

On the other hand, Fig. (2, d) shows the fourth order (4D) spectra of DV and SDM, and also their corresponding zero-crossing wavelengths were indicated in this figure, it was found that the chosen zero crossing wavelengths (289 nm for DV and 224 nm for SDM) have in front peaks with maximum or near to maximum amplitudes to the other drug, similar as in 3D method.

3.4.2. Linearity range

The calibration graphs were constructed under the selected optimal conditions using the value of derivative amplitude versus concentration of studied compound. The analysis of these graphs was made using least squares method for the slope, intercept and correlation coefficient, the limit of detection, limit of quantification, the correlation coefficients and the range of linearity, Table 1. For diaveridine, a linear correlation was obtained between peak amplitude and the corresponding concentration in the range of 2.5-35.0 µg/mL, from which the linear regression equation was computed and found to be:

$$\begin{aligned} {}^1D &= 0.0387 X + 0.0125 & \text{at } 243 \text{ nm,} & \quad r=0.9992 \\ {}^2D &= 0.041 X + 0.01 & \text{at } 235 \text{ nm,} & \quad r = 0.9992 \\ {}^3D &= 0.1215 X - 0.015 & \text{at } 244 \text{ nm} & \quad r=0.9994 \\ {}^4D &= 0.0317 X - 0.0312 & \text{at } 289 \text{ nm} & \quad r=0.9986 \end{aligned}$$

Where D: is the derivative amplitude value ($\Delta\lambda/\Delta A$), X: is the concentration of the drug in µg/mL and r: is the correlation coefficient. For sulfadimidine, the optimal concentration range was 1.0-25.0 µg/mL and the linear regression equation is found to be:

$$\begin{aligned} {}^1D &= 0.0282 X + 0.008 & \text{at } 258 \text{ nm,} & \quad r=0.9998 \\ {}^2D &= 0.029 X + 0.001 & \text{at } 273 \text{ nm,} & \quad r=0.9998 \\ {}^3D &= 0.0421 X - 0.021 & \text{at } 238 \text{ nm} & \quad r=0.9998 \\ {}^4D &= 0.034 X - 0.031 & \text{at } 224 \text{ nm} & \quad r=0.9986 \end{aligned}$$

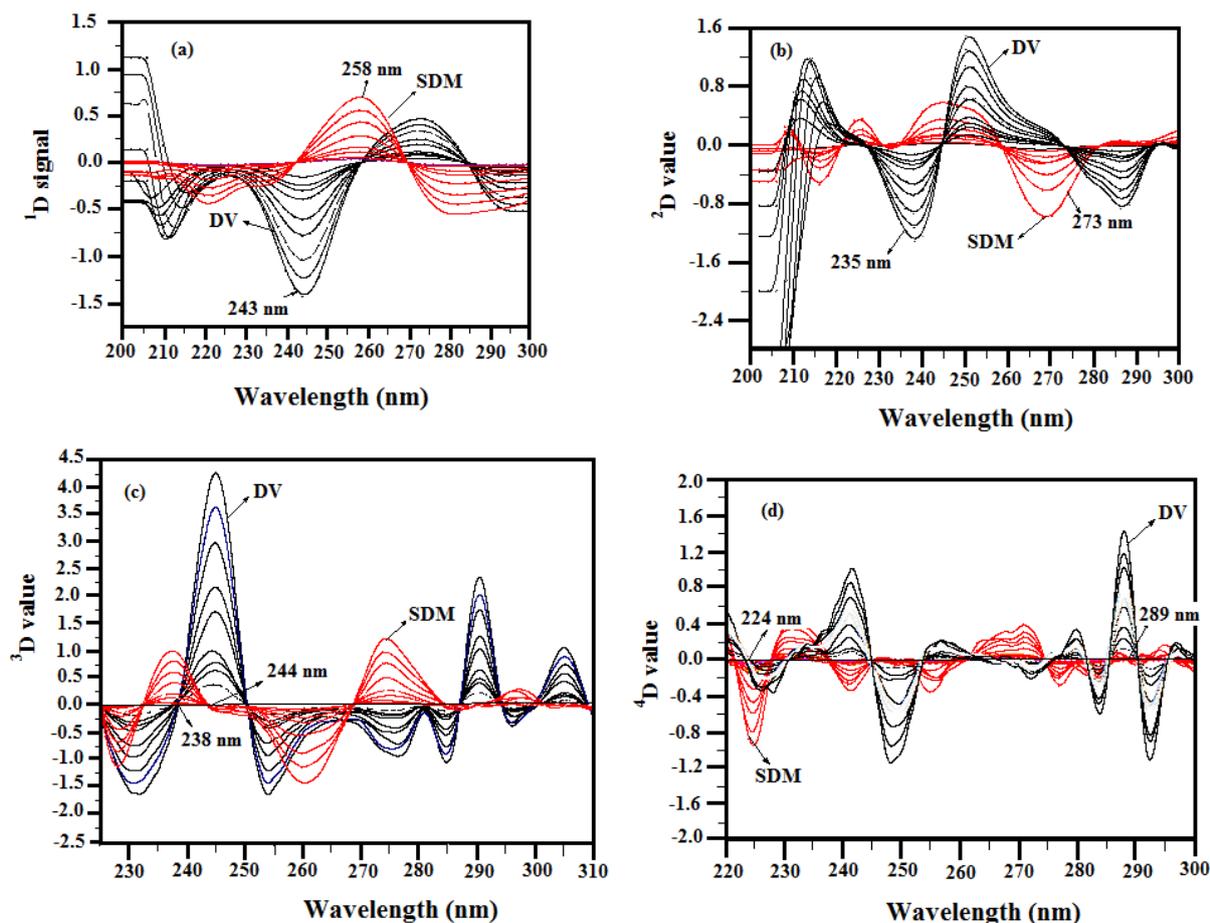


Fig. 2. (a) 1st, (b) 2nd, (c) 3rd and (d) 4th spectra for 2.5-35.0 µg/mL DV and 1.0-25.0 µg/mL SDM in methanol

Table 1: Validation of the results obtained by applying the ¹D, ²D, ³D and ⁴D methods.

Parameters	DV				SDM			
	¹ D	² D	³ D	⁴ D	¹ D	² D	³ D	⁴ D
Crossing point	242	235	244	289	258	273	238	224
Range (µg/mL)	2.5-35.0	2.5-35.0	2.5-35.0	2.5-35.0	1.0-25.0	1.0-25.0	1.0-25.0	1.0-25.0
Slope	0.0387	0.030	0.122	0.0317	0.028	0.029	0.042	0.034
Intercept	0.013	0.013	0.020	0.031-	0.008	-0.001	0.021-	0.031-
r ²	0.9991	0.9998	0.9994	0.9994	0.9979	0.9996	0.9989	0.9989
S.D.	0.048	0.030	0.031	0.020	0.036	0.027	0.044	0.026
LOD (µg/mL)	2.32	2.50	0.77	1.89	3.19	2.59	3.22	2.29
LOQ (µg/mL)	7.73	8.33	2.55	6.31	10.62	8.63	10.73	7.64
S.E.	0.234	0.268	0.204	0.1355	0.256	0.284	0.226	0.192

In order to estimate the precision (RSD) of the method, three replicate samples included the cited drugs at different concentration levels were measured

individually. It is interesting to observe the good

agreement between these measurements. The accuracy of the results was checked by applying the proposed methods for determination of different blind samples of pure diaveridine and sulfadimidine.

The concentrations were obtained from Beer's law. From which the percentage recoveries were calculated and shown in Table 2. The good recoveries suggested good accuracy of the proposed methods, in case of determination of diaveridine using 1D , 2D , 3D and 4D methods, the mean recovery percentage values were (100.45 for 1D), (100.64 for 2D), (100.33 for 3D) and (100.55 for 4D), from this we can deduce that 3D method is the most suitable for determination of diaveridine, on the other hand, in case of determination of sulfadimidine, the mean recovery percentage values were (100.52 for 1D), (100.63 for 2D), (100.48 for 3D) and (100.71 for 4D), it means that 3D method is also the most suitable for determination of sulfadimidine, Table 2. The recovery was calculated using the following equation:

$$\text{Recovery} = \frac{\text{Found concentration}}{\text{Taken concentration}} \times 100$$

3.5. Determination of diaveridine and sulfadimidine in laboratory prepared binary mixtures using 1D , 2D , 3D and 4D methods (selectivity)

The proposed method was used for determination of diaveridine in presence of sulfadimidine in different laboratory prepared mixtures without prior separation. For this purpose, a series of binary mixtures of diaveridine and sulfadimidine was prepared. The first one concentration level varied from 10-30 $\mu\text{g/mL}$, the second one varied in the range 5.0-25.0 $\mu\text{g/mL}$. In order to estimate the precision of the method and the homogeneity of the results, three replicate determinations of various binary mixtures of diaveridine and sulfadimidine were measured. The relative standard deviation values were calculated for each mixture. In order to estimate the accuracy of the method and reproducibility of the results, the recovery test was performed using the cited synthetic mixtures. The results were listed (Table 3), the results indicated that, in case of determination of diaveridine in laboratory prepared binary mixtures using 1D , 2D , 3D and 4D methods, the recovery values varied in range (100.33-100.66 for 1D), (100.35-100.88 for 2D), (99.92-100.50 for 3D) and (99.80-100.80 for 4D), from this we can deduce that 3D method is the most suitable for determination of diaveridine in binary mixtures, but in case of determination of sulfadimidine in the same binary mixtures, the recovery percent values varied in range (100.30-100.66 for 1D), (100.60-101.10 for 2D), (99.40-100.50 for 3D) and (99.80-100.70 for 4D), it means that 3D

method is also the most suitable for determination of sulfadimidine in binary mixtures.

3.6. Applications for determination of diaveridine and sulfadimidine in pharmaceutical preparation using the proposed methods

The applicability of the proposed methods has been tested on commercially available pharmaceutical formulations such as New-Cox[®] and Sulfadine-K[®]. The concentration of the drugs in the dosage forms was calculated from the appropriate calibration graphs. There was no shift in the working wavelengths or amplitude of the signals due to the presence of other constituents of the dosage forms. Table 4 shows the results obtained for the determination of diaveridine and sulfadimidine in the dosage forms. As can be seen, the agreement between calculated and experimental values is good; also we find that the recovery studies indicated that the excipients in the drug forms did not interfere in the determinations because these methods estimate each component independently. In addition, the estimated drugs content with low values of standard deviation further established the precision and the accuracy of the assaying methods. The results obtained were compared statistically by the Student's t-test (for accuracy) and the variance ratio F-test (for precision) with those obtained by the reported method [34, 35], Table 4. The values of t- and F-tests obtained at 95% confidence level did not exceed the theoretical tabulated value indicating no significant difference between the methods compared. From this we can deduce that, the developed methods may be recommended for routine and quality control analysis of the investigated drugs to provide simple, accurate and reproducible quantitative analysis for determination of diaveridine and sulfadimidine in bulk powder and pharmaceutical formulations.

Table 2: Accuracy of the determination of diaveridine or sulfadimidine by applying the proposed derivative methods.

Method	Taken Conc. µg/mL	<u>Diaveridine</u>		<u>Sulfadimidine</u>	
		Found Conc. µg/mL	Recovery %	Found Conc. µg/mL	Recovery %
¹ D	10	10.04	100.04	10.06	100.60
	15	15.12	100.80	15.05	100.33
	20	20.04	100.20	20.13	100.65
	25	25.10	100.40	25.12	100.48
	Mean	100.45		100.52	
	RSD%	0.47		0.43	
² D	10	10.08	100.80	10.06	100.60
	15	15.10	100.67	15.12	100.80
	20	20.13	100.65	20.11	100.55
	25	25.11	100.44	25.14	100.56
	Mean	100.64		100.63	
	RSD%	0.45		0.48	
³ D	10	10.02	100.20	10.05	100.50
	15	15.07	100.50	15.07	100.46
	20	20.06	100.30	20.09	100.45
	25	25.07	100.30	25.13	100.50
	Mean	100.33		100.48	
	RSD%	0.12		0.35	
⁴ D	10	10.06	100.60	10.08	100.80
	15	15.10	100.70	15.12	100.80
	20	20.09	100.50	20.14	100.70
	25	25.11	100.40	25.13	100.52
	Mean	100.55		100.71	
	RSD%	0.18		0.21	

Table 3: Analysis of laboratory prepared mixtures contains different concentration ratios of diaveridine and sulfadimidine for the determination of diaveridine and sulfadimidine using 1D , 2D , 3D and 4D methods

Taken Conc. $\mu\text{g/mL}$		1D		2D		3D		4D	
DV	SDM	Found Conc. $\mu\text{g/mL}$	Recovery %	Found Conc $\mu\text{g/mL}$	Recovery %	Found Conc. $\mu\text{g/mL}$	Recovery %	Found Conc. $\mu\text{g/mL}$	Recovery %
Diaveridine									
10	25	10.04	100.4	10.04	100.4	10.05	100.5	10.09	100.9
10	20	10.06	100.6	10.06	100.6	10.04	100.4	10.08	100.8
15	20	15.05	100.3	15.09	100.6	15.07	100.5	15.11	100.7
20	20	20.12	100.6	20.17	100.9	20.09	100.5	20.07	100.4
20	15	20.11	100.6	20.13	100.7	20.08	100.4	19.96	99.8
20	10	20.09	100.5	20.07	100.4	20.05	100.3	19.98	99.9
25	10	25.12	100.5	25.22	100.9	24.98	99.9	25.11	100.4
Sulfadimidine									
25	10	10.06	100.6	10.06	100.6	9.94	99.4	10.01	100.1
20	10	10.03	100.3	10.11	101.1	10.05	100.5	10.02	100.2
20	15	15.05	100.3	15.14	100.90	15.08	100.5	15.04	100.3
20	20	20.07	100.4	20.19	101.0	19.97	99.9	20.14	100.7
15	20	20.10	100.5	20.16	100.8	20.07	100.4	20.06	100.3
10	20	20.08	100.4	20.10	100.5	20.09	100.5	20.09	100.5
10	25	25.13	100.5	25.18	100.7	25.11	100.4	24.95	99.8

3.7. First derivative ratio spectra (1DD) method for the simultaneous determination of diaveridine and sulfadimidine in a binary mixture

Ratio spectra derivative spectrophotometry offers greater selectivity than the conventional spectrophotometry for simultaneous determination of the two drugs in a mixture without previous chemical separation; it also has a great ability to resolve mixture of compounds exhibiting highly overlapped spectra successfully with high selectivity and accuracy. The absorption spectrum of the mixture is obtained and divided (amplitude by amplitude at appropriate wavelengths) by the absorption spectrum of a standard solution of one of the components (previously stored in a computer), and the derivative of the ratio spectrum is obtained.

In this part, first derivative of ratio spectrophotometric method (1DD) was proposed for the simultaneous determination of sulfadimidine and

diaveridine in a binary mixture and to eliminate any spectral overlap with high selectivity, sensitivity and accuracy, the ratio spectra of diaveridine was obtained by dividing its absorption spectra by the absorption spectrum of a certain concentration of sulfadimidine. Similarly, the ratio spectra of sulfadimidine had been obtained by dividing its absorption spectra by the absorption spectrum of a certain concentration of diaveridine and then the first derivative of the obtained ratio spectra of sulfadimidine and diaveridine, separately, can be obtained easily.

To optimize the 1DD method for determination of diaveridine and sulfadimidine in their binary mixture we have first study the influence of certain variables on the derivative ratio spectra such as: divisor concentration, $\Delta\lambda$ and scaling factor (SF).

3.7.1. The effect of divisor concentrations

The stored spectra of working standard solution of diaveridine (2.5-35 $\mu\text{g/mL}$) were divided several times by the spectrum of working standard solution

of sulfadimidine each time with different concentrations (2.0, 4.0, 6.0, 8.0 and 10.0 $\mu\text{g/mL}$) in order to obtain the most suitable concentration used as a divisor in the determination of diaveridine ratio spectra. The second divisor (4.0 $\mu\text{g/mL}$) was found the best regarding it has a good signal to noise ratio with good slope and intercept values and more repeatable results. Fig. (3, A) shows ratio spectra of different concentrations of DV (2.5-35.0 $\mu\text{g/mL}$) divided by zero-order absorption spectrum of SDM (4.0 $\mu\text{g/mL}$).

plotted against the corresponding concentrations of diaveridine, the statistical analyses of these calibration graphs were obtained using least squares method. Similarly, the stored spectra of the working standard solutions of sulfadimidine (1.0-25.0 $\mu\text{g/mL}$) were divided several times by the spectrum of working standard solution of diaveridine each time with different concentrations (1.0, 2.5, 5.0, 7.5 and 10.0 $\mu\text{g/mL}$) in order to obtain the most suitable concentration used as a divisor in the determination of sulfadimidine ratio spectra, It was found that the first derivative of ratio spectra (1DD) method was

Table 4: The analysis of diaveridine^a and sulfadimidine^b in pharmaceutical dosage forms by the proposed ¹D, ²D, ³D and ⁴D methods

Dosage Forms	Taken $\mu\text{g/mL}$	¹ D		² D		³ D		⁴ D	
		Found* $\mu\text{g/mL}$	Recovery%						
New-Cox ^a	10	10.16	101.6	9.82	98.2	10.14	101.4	10.16	101.6
	15	15.15	101.0	14.88	99.2	15.17	101.1	15.15	101.0
	20	20.16	100.8	19.88	99.4	20.15	100.8	20.16	100.8
t-test		1.06		0.97		1.06		2.01	
F-test		1.78		1.54		1.78		3.19	
Sulfadine-K ^b	10	10.27	102.7	10.29	102.9	10.09	100.9	10.07	100.7
	15	15.26	101.7	15.26	101.7	15.04	101.3	15.09	100.6
	20	20.26	101.3	20.25	101.3	20.12	101.6	20.16	100.8
t-test		2.04		1.77		2.04		1.94	
F-test		2.21		2.96		2.12		3.24	
Entroqeen ^b	10	10.25	102.5	10.26	102.6	9.95	99.5	10.15	101.5
	15	14.82	98.8	15.21	101.4	14.82	98.8	15.17	101.1
	20	20.25	101.25	19.87	99.4	19.89	99.5	20.15	100.8
t-test		1.15		1.29		1.96		2.03	
F-test		2.13		3.24		2.25		2.52	

The first derivative then was applied under certain selected instrumental parameters $\Delta\lambda=4$, scaling factor SF=1 and wavelength range (220-350 nm). The peak amplitudes were measured and their values were

applied by choosing the concentration of 7.5 $\mu\text{g/mL}$ as convenient divisor concentration where it has a good signal to noise ratio with good slope and

intercept values and more repeatable results, the curves are shown in Fig. (3, B).

The first derivative then was applied under certain selected instrumental parameters $\Delta\lambda = 8$, scaling factor $SF = 1$ and wavelength range (220-350 nm). The peak amplitudes were measured and their values were plotted against the corresponding concentrations of sulfadimidine, the statistical analyses of these calibration graphs were obtained using least squares method.

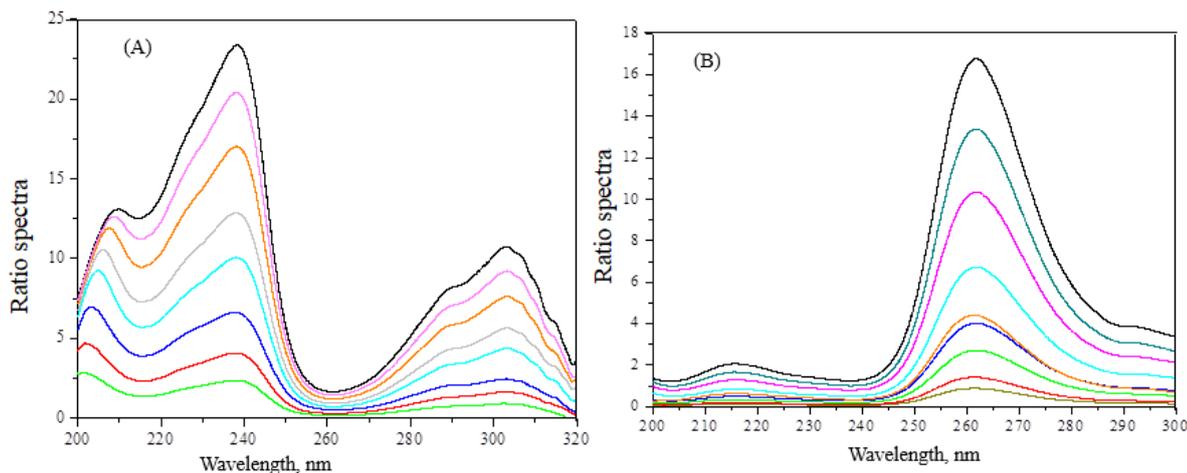


Fig 3. Ratio spectra of different concentrations of DV (2.5-35.0 $\mu\text{g/mL}$) divided by zero-order absorption spectrum of SDM (4.0 $\mu\text{g/mL}$), (A) and ratio spectra of different concentrations of SDM (2.5-35.0 $\mu\text{g/mL}$) divided by zero-order absorption spectrum of DV (7.5 $\mu\text{g/mL}$), (B).

3.7.2. Selection of analytical wavelengths

Figure (4) shows the first derivative ratio (^1DD) spectra of diaveridine and sulfadimidine, also their corresponding zero-crossing wavelengths were indicated in these figures. These wavelengths achieved the more accurate, reproducible and sensitive results, so they would be examined to be the optimum working wavelengths for the simultaneous determination of diaveridine and sulfadimidine in binary mixtures using the first derivative ratio (^1DD) method.

3.7.3. Linearity range

Under the experimental conditions, by applying 1DD method, calibration curves were constructed by

plotting the peak amplitudes at the selected wavelengths versus concentration of the studied drugs.

For diaveridine, a linear correlation was obtained between peak amplitude and the corresponding concentration in the range of 2.5-35.0 $\mu\text{g/mL}$, where that for sulfadimidine, was 1.0-25.0 $\mu\text{g/mL}$.

The validation of ^1DD method for the determination of diaveridine and sulfadimidine in a binary mixture was applied by choosing the working

wavelengths (223 and 245 nm for DV and 254 and 271 nm for SDM), where they have the most suitable values of the regression equations parameters, the analytical data at these wavelengths were grouped in Table 5, the limit of detection and limit of quantification were determined according to ICH recommendations [34] which are also grouped in Table 5.

Table 5 : Validation of the results obtained by applying the first derivative ratio method (^1DD).

Parameters	Diaveridine		Sulfadimidine	
	$\lambda_{223 \text{ nm}}$	$\lambda_{245 \text{ nm}}$	$\lambda_{254 \text{ nm}}$	$\lambda_{271 \text{ nm}}$
Range ($\mu\text{g/mL}$)	2.5-35	2.5-35	1-25	1-25
Slope	0.059	-0.063	0.045	-0.028
Intercept	-0.009	-0.18	0.009	-0.007
(r)	0.9996	0.9989	0.9996	0.9994
S.D.	0.039	0.024	0.036	0.032
LOD ($\mu\text{g/mL}$)	1.97	1.142	2.35	3.24
LOQ ($\mu\text{g/mL}$)	6.58	3.81	7.83	11.42
S.E.	0.198	0.209	0.188	0.212

3.7.4. Determination of diaveridine and sulfadimidine in bulk powder using ¹DD method

The accuracy of the proposed method was determined by investigation the percentage recovery at four concentration levels, each in triplicate, ranging from 10.0-25.0 µg/mL for both drugs. ¹DD values were measured at 223 and 254 nm, for diaveridine and sulfadimidine, respectively. The concentrations were obtained from Beer's law. The percentage recoveries were calculated and shown in Table 6. In determination diaveridine and sulfadimidine the mean recovery value was 100.3%. The obtained results indicated the agreement between them and those accepted as true. The good recoveries suggested good accuracy of the proposed methods.

Table 6: Analysis of laboratory prepared mixtures contains different concentration ratios of DV and SDM for the determination of diaveridine using 1DD method

Taken Conc. µg/mL			DV		SDM	
			Found Conc. µg/mL	Recovery %	Found Conc. µg/mL	Recovery %
DV	SDM	DV%	µg/mL		µg/mL	
10	25	40	10.05	100.5	10.04	100.4
10	20	50	10.03	100.3	10.07	100.7
15	20	75	20.07	100.4	15.09	100.6
20	20	100	20.06	100.3	19.98	99.9
20	15	133	19.96	99.8	20.04	100.2
20	10	200	19.93	99.7	20.06	100.3
25	10	250	24.97	99.9	25.05	100.2

3.7.5 Determination of diaveridine and sulfadimidine in laboratory prepared binary mixtures using ¹DD method (selectivity).

The proposed method used for determination of diaveridine in presence of sulfadimidine in different laboratory prepared mixtures without prior separation. For this purpose, a series of binary mixtures of diaveridine and sulfadimidine was prepared. The concentration level of the two drugs varied from 10-25 µg/mL, ¹DD values were measured at 223 and 254 nm, for diaveridine and sulfadimidine, respectively. Then the concentrations of diaveridine and sulfadimidine were calculated from the corresponding regression equations. In order to estimate the precision of the method and the homogeneity of the results, three replicate determinations of various binary mixtures of diaveridine and sulfadimidine were measured. The relative standard deviation values were calculated for

each mixture. In order to estimate the accuracy of the method and reproducibility of the results, the recovery values% were calculated for the cited synthetic mixtures. The results were listed at Table 6.

3.7.5 Applications for determination of diaveridine and sulfadimidine in pharmaceutical preparation using ¹DD method).

The applicability of the proposed methods has been tested on commercially available pharmaceutical formulations such as New-Cox[®] and Sulfadine-K[®], three replicate determinations were made. ¹DD values were measured at 223 and 254 nm, for diaveridine and sulfadimidine, respectively. Satisfactory results were obtained for diaveridine and sulfadimidine and were in a good agreement with label claims. The results obtained were compared statistically by the Student's t-test (for accuracy) and the variance ratio F-test (for precision) with those obtained by the reported method [35, 36], Table 7. The values of t- and F-tests obtained at 95% confidence level did not exceed the theoretical tabulated value 2.306 and 6.39 indicating no significant difference between the performance of these methods regarding their accuracy and precision.

However, the derivative spectrophotometry technique was adopted in order to determine binary mixture of constituents, because it is a reliable technique which does not require complex processing of mathematical data. Therefore, it is much easier to apply than the numerical type method; the investigated methods were further validated by estimation of diaveridine and sulfadimidine in different pharmaceutical formulations, there is no interference from the formulation matrix present in the studied formulations.

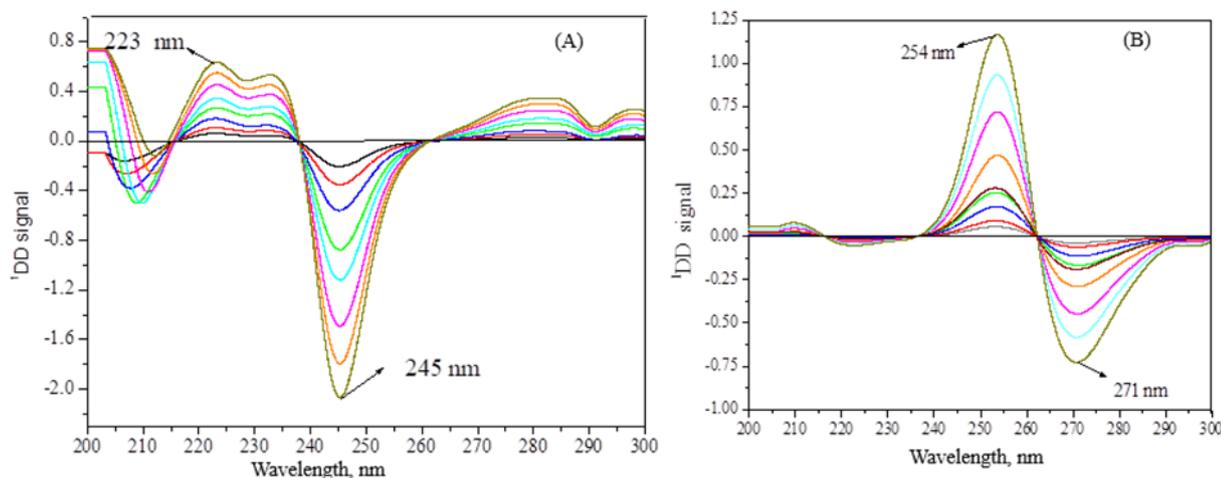


Fig. 4. First-derivative ratio spectra for DV (2.5-35.0 $\mu\text{g/mL}$) (A), and for SDM (1.0-25.0 $\mu\text{g/mL}$) (B) in methanol.

Table 7: The analysis of diaveridine^a and sulfadimidine^b in pharmaceutical dosage forms by the proposed ¹DD method.

Pharmaceutical dosage forms	Taken ($\mu\text{g/mL}$)	Found* ($\mu\text{g/mL}$)	Recovery%	Relative error (%)	t-test	F-test
New -Cox^{®a}	10	10.07	100.70	0.70		
	15	15.08	100.50	0.50	0.69	0.52
	20	20.05	100.25	0.25		
Sulfadine-K^{®b}	10	10.07	100.70	0.90		
	15	15.03	100.20	0.26	1.14	1.87
	20	20.09	100.45	0.60		
Entroqueen^{®b}	10	10.01	100.10	0.50		
	15	15.07	100.47	1.20	0.98	1.07
	20	20.04	100.20	0.55		

4. Conclusions

The main task of this study is to find fast, accurate, sensitive and cost effective spectrophotometric techniques for the simultaneous determination of diaveridine and sulfadimidine in pure forms and in some commercial pharmaceutical

preparations. It is based on derivative spectrophotometry, first, second, third, fourth and first ratio derivative spectra of binary mixtures containing diaveridine and sulfadimidine were established. The results were in a good linearity with negligible intercepts, the high values of correlation coefficients indicate the good linearity of all calibration graphs and the validity of Beer's law to

derivative measurements. Satisfactory results were obtained for the recovery of each drug in the mixtures. From recovery studies, ¹DD and ³D methods are the most suitable for determination of sulfadimidine and diaveridine in binary mixtures. The proposed methods are selective and sensitive for simultaneous determination of the drugs.

5. Conflicts of interest

There is no conflict of interest.

6. Formatting of funding sources

No funding sources

7. References

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