

## COMPARATIVE STUDY AMONG FOUR SPECTROPHOTOMETRIC METHODS FOR THE SIMULTANEOUS DETERMINATION OF BINARY MIXTURE OF CHLORZOXAZONE AND DICLOFENAC POTASSIUM

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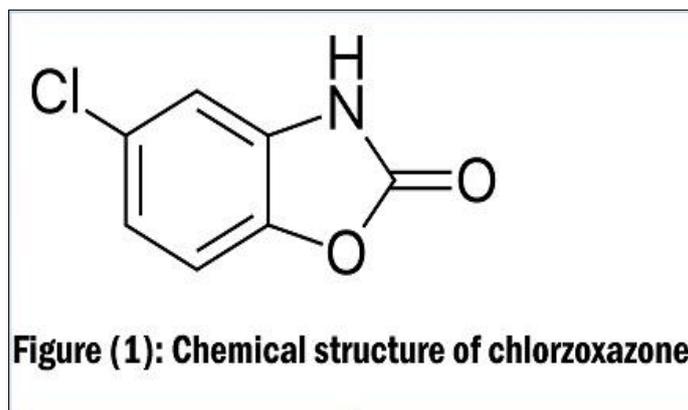
### ABSTRACT

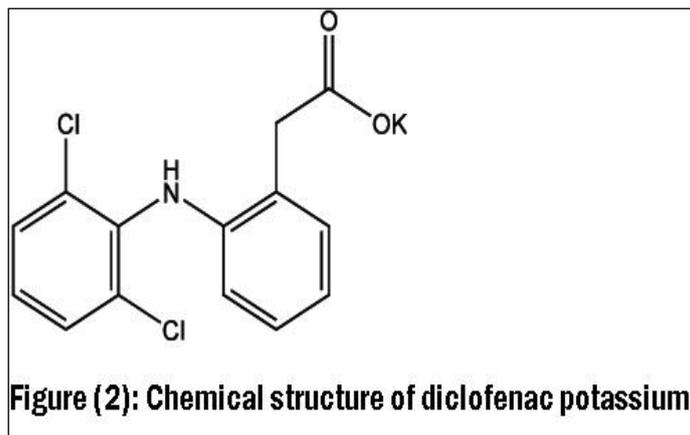
Four simple, fast, accurate, reproducible, and non-sophisticated spectrophotometric methods were developed and validated for the simultaneous determination of chlorzoxazone and diclofenac potassium without preliminary separation in pure powder form and in their capsule formulation. Method A, is a dual wavelength spectrophotometric method in which the wavelengths selected for determination of chlorzoxazone were 259 nm and 294 nm, whereas the wavelengths selected for determination of diclofenac potassium were 294 nm and 242 nm. while method B, is a ratio difference spectrophotometric method in which the wavelengths selected for determination of chlorzoxazone were 280 nm and 230 nm, whereas the wavelengths selected for determination of diclofenac potassium were 246 nm and 284 nm. while method C, is the first derivative of the ratio spectra measured at 290 nm and 301 nm for chlorzoxazone and diclofenac potassium, respectively. While method D, is the constant center spectrophotometric method in which more measured at 280 nm and 277 nm for chlorzoxazone and diclofenac potassium, respectively. Regression analysis of Beer-Lambert's plots showed good correlation in concentration range of 2.5 – 20 µg/mL for both drugs with LOD 0.308 µg/mL and 0.932 µg/mL, LOQ 0.458 µg/mL and 1.388 µg/mL, RSD 1.325 and 1.666 and % recovery 98.99 and 101.23 for chlorzoxazone and diclofenac potassium, respectively in method A. Furthermore, LOD 0.307 µg/mL and 0.373 µg/mL, LOQ 0.929 µg/mL and 1.123 µg/mL, RSD 1.065 and 1.371 and % recovery 99.94 and 101.53 for chlorzoxazone and diclofenac potassium, respectively in method B. Furthermore, LOD 0.287 µg/mL and 0.301 µg/mL, LOQ 0.871 µg/mL and 0.911 µg/mL, RSD 1.214 and 1.596 and % recovery 99.73 and 100.26 for chlorzoxazone and diclofenac potassium, respectively in method C. Furthermore, LOD 0.221 µg/mL and 0.331 µg/mL, LOQ 0.521 µg/mL and 0.825 µg/mL, RSD 0.914 and 1.412 and % recovery 101.22 and 98.59 for chlorzoxazone and diclofenac potassium, respectively in method D. The suggested methods were validated in compliance with the ICH guidelines and were successfully applied for determination of chlorzoxazone and diclofenac potassium in their laboratory prepared mixtures and commercial capsule formulation.

**Keywords:** Dual wavelength; Ratio difference; Ratio derivative; Constant center; Chlorzoxazone; Diclofenac potassium; Binary mixture.

**Introduction:**

Chlorzoxazone (CHZ) **figure (1)**, chemically known as 5-chloro-2-benzoxazolinone, is a centrally acting skeletal muscle relaxant with sedative properties, and it is official in the United States Pharmacopeia [U.S. Pharmacopoeia,2011]. It is claimed to inhibit muscle tone and tension and, thus, inhibit spasm and pain by an effect primarily at the level of the spinal cord by depressing reflexes and subcortical areas of the brain [Badgujar MA, etal,2011]. Diclofenac potassium (DIC) **figure (2)**, chemically known as 2-[(2,6-dichlorophenyl) amino] benzene acetic acid, monopotassium, is a non-steroidal anti-inflammatory drug (NSAID), and it is official in the British Pharmacopoeia [B. Pharmacopoeia,2012]. It has analgesic and antipyretic actions, It is a potent inhibitor of prostaglandin biosynthesis by inhibition of both leukocyte migration and (COX-1 and COX-2) cyclooxygenase enzymes [Patel RA,etal,2014. Panchumarthy R,etal,2013]. It is used in the treatment of signs and symptoms of osteoarthritis and rheumatoid arthritis, and it has been shown to be effective in relieving headache in migraine attacks [Panchumarthy R,etal,2013]. The combination of CHZ and DIC is indicated for the treatment, prevention, control, and improvement of muscle aches, pain and swelling, dental pain, back pain, sports injuries, menstrual cramps, and other conditions by reducing the substances in the body that cause inflammation and pain and by blocking pain sensations that are sent to the brain [Badgujar MA, etal,2011. Patel RA,etal,2014. Panchumarthy R,etal,2013]. However, the literature review revealed that few studies have estimated chlorzoxazone and diclofenac potassium simultaneously by HPLC [Ahmed HM,etal,2020. Krutika P,etal,2021. Sakinala P,etal,2020], HPTLC [Ahmed HM,etal,2020] and spectrophotometric methods [Hegazy MA,etal,2018]. So, we aimed in this presented work to develop four simple spectrophotometric methods for the simultaneous analysis of the binary mixture of CHZ and DIC in their laboratory prepared mixtures and commercial capsule formulation.





## 2. Experimental:

### 2.1. Apparatus and Software:

Shimadzu-UV 1800 double beam UV-Visible spectrophotometer (Japan) with matched 1 cm quartz cells at 200 - 800 nm range was used for all absorbance measurements. Spectra were automatically obtained by Shimadzu UV-Probe 2.32 system software.

### 2.2. Chemicals and Reagents:

#### 2.2.1 Pure Standard:

CHZ and DIC were received as a gift from Pharco Pharmaceuticals (Alexandria, Egypt) with claimed purities of 99.90% and 100.10%, respectively, according to manufacturer certificates of analysis.

#### 2.2.2 Pharmaceutical Formulation:

Declophen plus<sup>®</sup> capsules (batch No. 210102) was manufactured by Pharco Pharmaceuticals (Alexandria, Egypt) and claimed to contain 250 and 50 mg CHZ and DIC, respectively, per capsule were purchased from local market.

#### 2.2.3 Chemicals and Reagents:

Methanol, analytical grade (El-Nasr Company, Egypt) was purchased .

### 2.3. Standard Solutions:

Standard stock solutions of CHZ (1 mg/mL) and DIC (1 mg/mL) were prepared by dissolving 100 mg of pure powders into 50 mL methanol and the volume was completed to 100 mL in a volumetric flask with methanol, separately. Working solutions (100 µg/mL) of each standard solution were acquired by transfer 10-mL from standard stock solution into 100-mL volumetric flask then complete the volume to the mark with methanol.

Aliquots (0.25 - 2 mL) of CHZ and DIC working solutions (100  $\mu\text{g}/\text{mL}$ ) were transferred into a series of 10 mL volumetric flasks, and the volume was completed to the mark with methanol.

## **2.4. Procedures:**

### **2.4.1. Linearity (construction of calibration curves):**

#### **Spectral characteristics:**

The absorption spectra were measured for the prepared solutions at (200 - 400 nm) against methanol as a blank.

#### **Dual wavelength method:**

Different aliquots equivalent to 2.5-20 $\mu\text{g}/\text{mL}$  CHZ and DIC, were transferred from their respective standard working solutions (100  $\mu\text{g}/\text{mL}$ ) into two separate series of 10-mL volumetric flasks and the volume was then completed using methanol. The absorption spectra of the prepared solutions were scanned (200–400nm) and stored in the computer. the absorbance values at 259 and 294 nm (for CHZ) and at 294 and 242 nm (for DIC) were measured. CHZ was determined by plotting the difference in absorbance values at 259 and 294 nm (difference is zero for DIC) against its corresponding concentration. Similarly for determination of DIC, the difference in absorbance values at 294 and 242 nm (difference is zero for CHZ) was plotted against the corresponding concentrations.

#### **Ratio difference method:**

The stored spectra were divided by the absorption spectra of 10  $\mu\text{g}/\text{mL}$  DIC and CHZ, respectively where the obtained ratio spectra were recorded. The amplitudes of the ratio spectra were measured at 280 and 230 nm for CHZ and 246 and 284 nm for DIC. Calibration curves relating the differences in amplitudes of the ratio spectra ( $\Delta P_{280 - 230}$ ) and ( $\Delta P_{246 - 284}$ ) to the corresponding concentrations were constructed and the regression equations were computed for CHZ and DIC, respectively.

#### **Ratio derivative method:**

The first derivative of the stored ratio spectra were recorded at ( $\Delta\lambda= 4$ ; scale 10) and ( $\Delta\lambda= 5$ ; scale 10) for CHZ and DIC, respectively. Calibration curves relating the  $^1\text{DD}$  amplitudes at 290 and 301 nm to the corresponding concentrations were constructed and the regression equations were computed for CHZ and DIC, respectively.

#### **Constant center method:**

Construct two calibration curves relating the absorbance of the zero order spectra of CHZ at 280 nm versus the corresponding concentrations of CHZ and DIC at

277 nm versus the corresponding concentrations of DIC, and then the regression equations were computed.

#### **2.4.2. Application to laboratory prepared mixtures:**

##### **Dual wavelength method:**

Zero order absorption spectra of different laboratory prepared mixtures containing different ratios of CHZ and DIC were recorded using methanol as blank and the procedure under linearity was then followed. Concentrations of CHZ and DIC in the prepared samples were calculated from the computed regression equations.

##### **Ratio difference method:**

The absorption spectra of different laboratory prepared mixtures were divided by the absorption spectra of 10 µg/ mL CHZ and DIC, respectively. The ratio spectra were recorded at 280 nm and 230 nm, 246 nm and 284 nm for CHZ and DIC, respectively. The concentrations of the drugs were calculated from the computed regression equations.

##### **Ratio derivative method:**

The absorption spectra of different laboratory prepared mixtures were divided by the absorption spectra of 10 µg/ mL CHZ and DIC, respectively. Proceed as detailed under linearity for both drugs. The concentration of each drug was calculated using the corresponding regression equation.

##### **Constant center method:**

The absorption spectra of different laboratory prepared mixtures were divided by the absorption spectra of 10 µg/ mL CHZ and DIC, respectively. The ratio spectra of CHZ and DIC were recorded at (246 nm and 284 nm) and (280 nm and 230 nm), respectively. The postulated amplitudes at 246 nm and 284 nm for CHZ and at 280 nm and 230 nm for DIC were calculated using the corresponding regression equations and the constant values were obtained after subtracting the recorded amplitudes of the mixtures and its postulated amplitudes at the specified wavelength (246 nm for CHZ and 280nm for DIC). Multiply the obtained constant values of CHZ and DIC for each mixture by the spectra of 10 µg/ mL CHZ and DIC, respectively, so the original spectra of CHZ and DIC are obtained. The concentrations of the drugs were calculated from the computed regression equations representing the absorbance of CHZ and DIC at 280 nm and 277 nm versus their concentrations, respectively.

#### **2.4.3. Application to pharmaceutical preparation:**

The content of ten Declophen plus<sup>®</sup> capsules was separately emptied, mixed, and weighed, and an amount of this formulation powder equivalent to 0.25 g CHZ and 0.05 g DIC was accurately transferred to a 100-mL volumetric flask. An amount of 75 mL of methanol was added, and the solution was sonicated for 15 minutes and allowed to cool

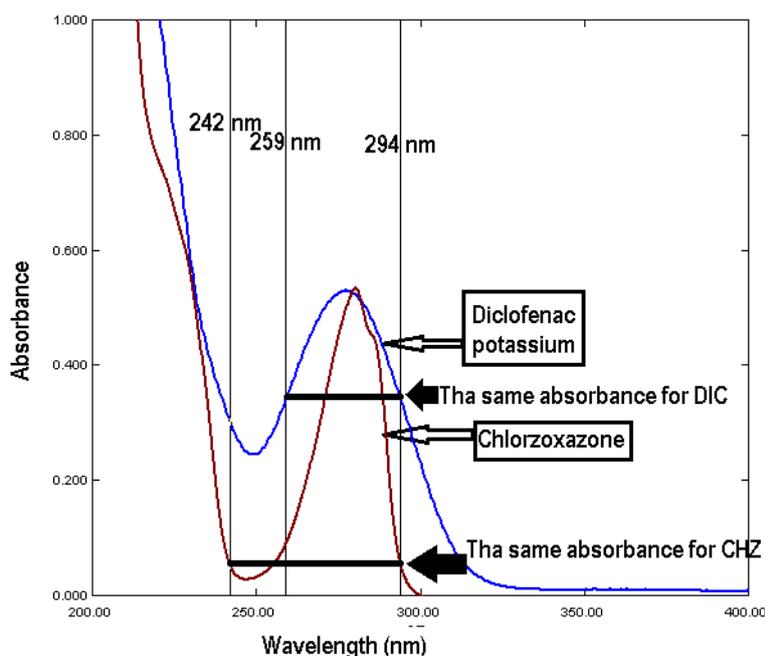
well, and then, the volume was adjusted to 100 mL with methanol and then filtered to prepare stock solutions of 2.5 mg/mL CHZ and 0.5 mg/mL DIC. The working sample solutions and different concentrations of CHZ and DIC were then prepared by suitable dilutions with methanol, and then, the procedures were followed under the analysis of laboratory prepared mixtures for each method and the concentrations of the cited drugs were calculated from the corresponding regression equations. When carrying out the standard addition technique, different known concentrations of pure standard CHZ and DIC were added to the pharmaceutical dosage form before proceeding in the proposed methods.

### 3. Results and discussions:

The aim of this work was to develop simple, sensitive, selective and precise spectrophotometric methods for the simultaneous determination of the components in binary mixtures with overlapping spectra without previous separation. The binary mixture of CHZ and DIC was chosen as an example for the application.

In the presented work, UV spectrophotometric methods; dual wavelength, ratio difference, ratio derivative and constant center spectrophotometric methods have been applied for the simultaneous determination of CHZ and DIC in their laboratory prepared mixtures and commercial capsule formulation.

The zero order absorption spectra of CHZ and DIC showed severe overlap which does not permit direct determination of the drugs, as shown in **figure (3)**.



**Figure (3) Zero-order absorption spectra of chlorzoxazone (10  $\mu\text{g/mL}$ ) and diclofenac potassium (10  $\mu\text{g/mL}$ ).**

### 3.1. Dual wavelength spectrophotometric method [Fernandes N, etal,2008]:

This method provides a simple method for selective determination of both CHZ and DIC using their zero order absorption spectra. The principle of this method is that the absorbance difference at two points on the spectra is directly proportional to the component of interest, independent of the interfering component. The pre-requisite for this method is the selection of two wavelengths where the interfering component shows the same absorbance value while the component of interest shows significant difference in absorbance with concentration. Selection of the suitable wavelengths plays an important role; hence different wavelengths were tried such as 243, 297 and 254, 298 nm for CHZ and 256, 294 and 235, 271 nm for DIC. Using the absorbance values at 259 and 294 nm (where DIC has the same absorbance) gave the best selectivity when used for determination of CHZ. On the other hand, absorbance values at 294 and 242 nm were chosen for determination of DIC where the best results were obtained. Calibration curves for CHZ and DIC were constructed by plotting the difference in absorbance values at the selected wavelengths for each drug against their corresponding concentrations as shown in **figures (4&5)**. CHZ and DIC obeyed Beer-Lambert's law in the concentration ranges of 2.5-20  $\mu\text{g/mL}$  for both drugs with good correlation coefficients. Regression equations as follows:  $Y = 0.0027X + 0.0094$  for CHZ and  $Y = 0.003X + 0.0077$  for DIC. Regression parameters are given in **table (1)**.

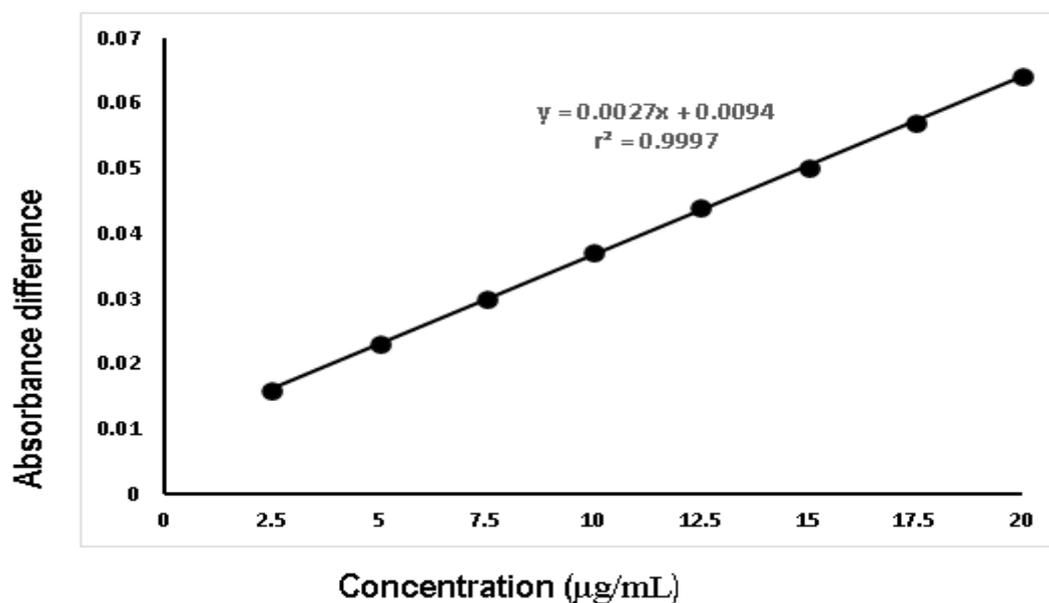
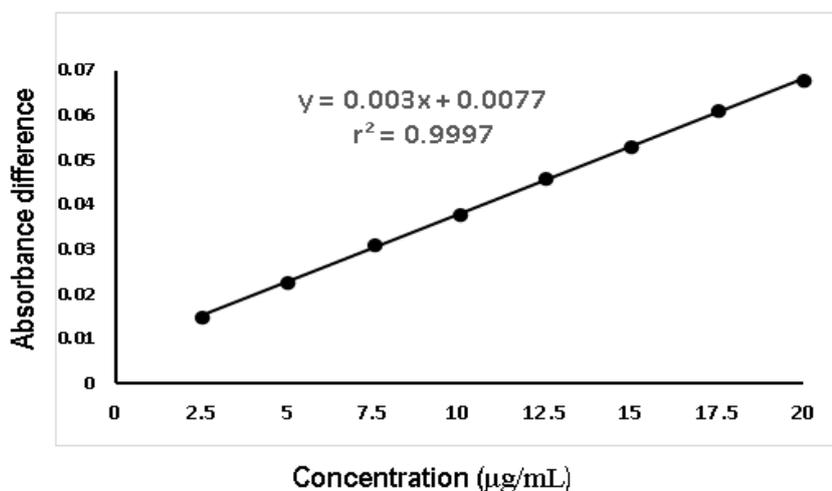


Figure (4) Calibration graph of chlorzoxazone by the proposed dual wavelength method.

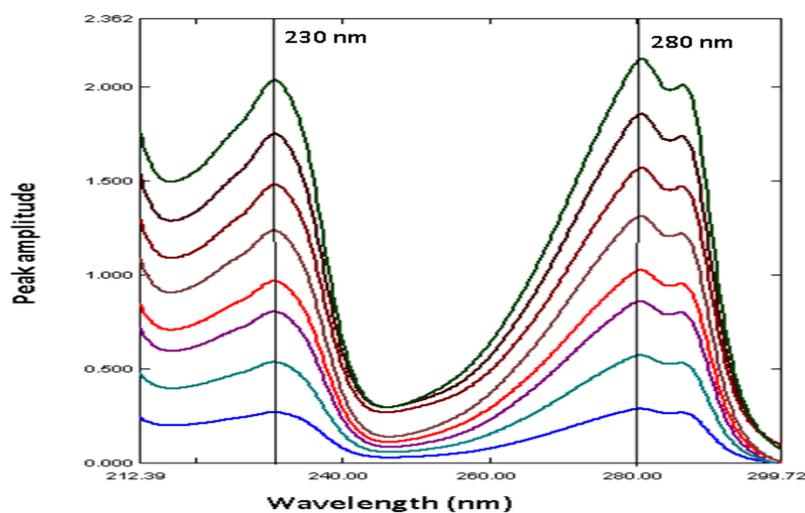


**Figure (5) Calibration graph of diclofenac potassium by the proposed dual wavelength method.**

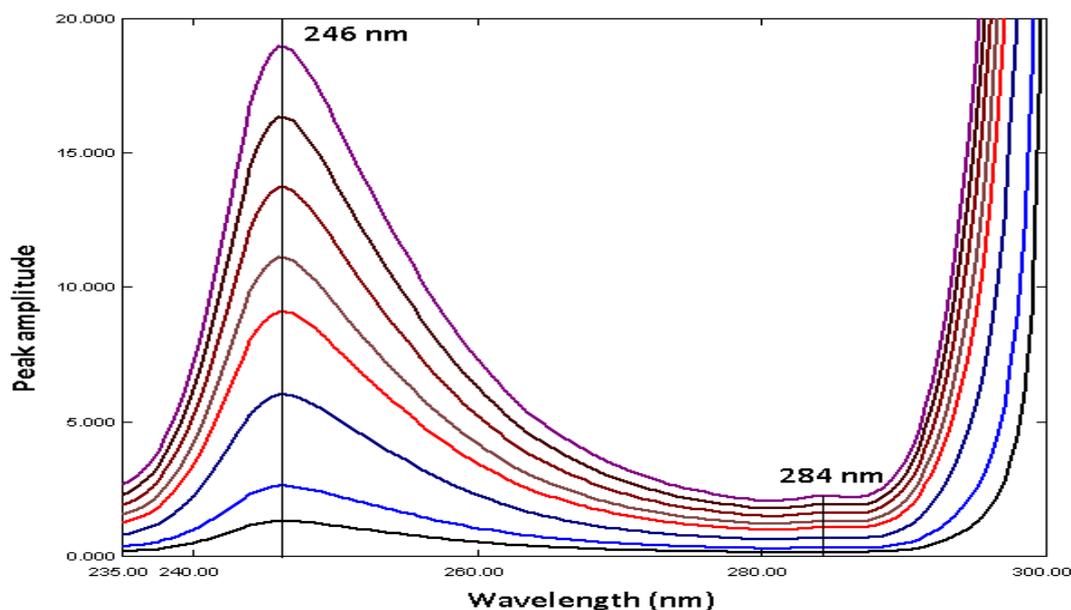
### 3.2. Ratio difference spectrophotometric method [Lotfy HM,etal,2012]:

For CHZ, the absorption spectra of the drug were divided by the absorption spectrum of DIC (10 µg/mL), as a divisor, to get the ratio spectra, as shown in **figure (6)**. The difference in peak amplitudes between 280 and 230 nm in the ratio spectra is proportional to the concentration of the drug without interference from DIC (divisor).

For DIC, the absorption spectra of the drug were divided by the absorption spectrum of CHZ (10 µg/mL), as a divisor, to get the ratio spectra, as shown in **figure (7)**. The difference in peak amplitudes between 246 and 284 nm in the ratio spectra is proportional to the concentration of the drug without interference from CHZ (divisor).

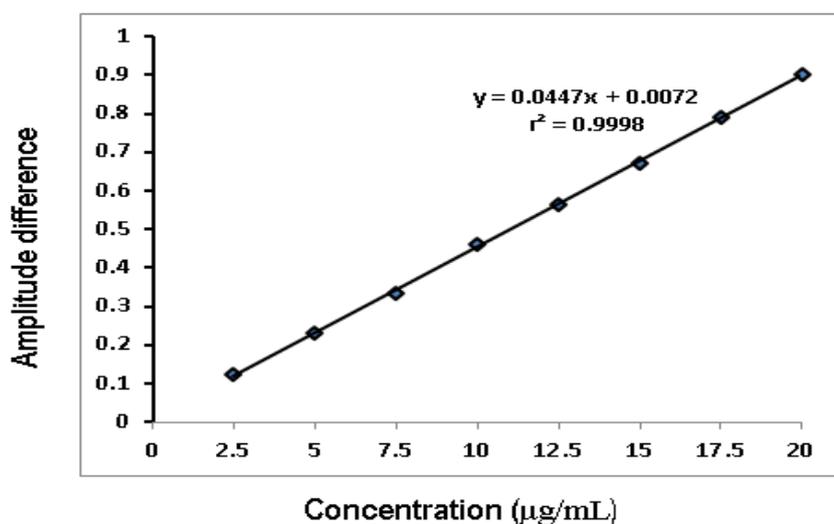


**Figure (6) Ratio spectra of chlorzoxazone at various concentrations (2.5 - 20 µg/mL) using 10 µg/mL of diclofenac potassium as a divisor.**

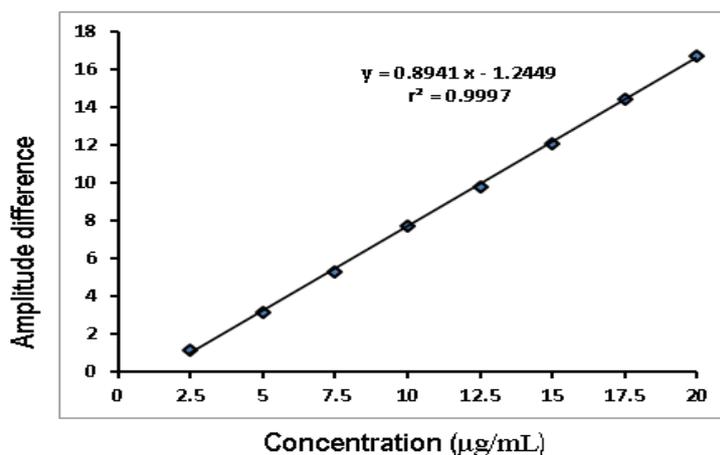


**Figure (7) Ratio spectra of diclofenac potassium at various concentrations (2.5 - 20 µg/mL) using 10 µg/mL of chlorzoxazone as a divisor.**

Calibration curves for CHZ and DIC were constructed by plotting the difference in amplitude values at the selected wavelengths for each drug against their corresponding concentrations as shown in **figures (8&9)**. CHZ and DIC obeyed Beer-Lambert's law in the concentration ranges of 2.5-20 µg/mL for both drugs with good correlation coefficients. Regression equations as follows:  $Y = 0.0447X + 0.0072$  for CHZ and  $Y = 0.8941X + 1.2449$  for DIC. Regression parameters are given in **table (1)**.



**Figure (8) Calibration graph of chlorzoxazone by the proposed ratio difference method.**

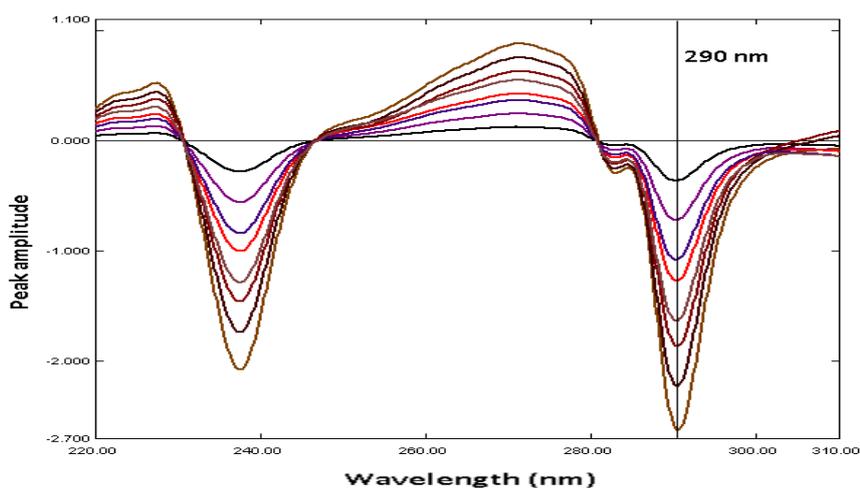


**Figure (9)** Calibration graph of diclofenac potassium by the proposed ratio difference method.

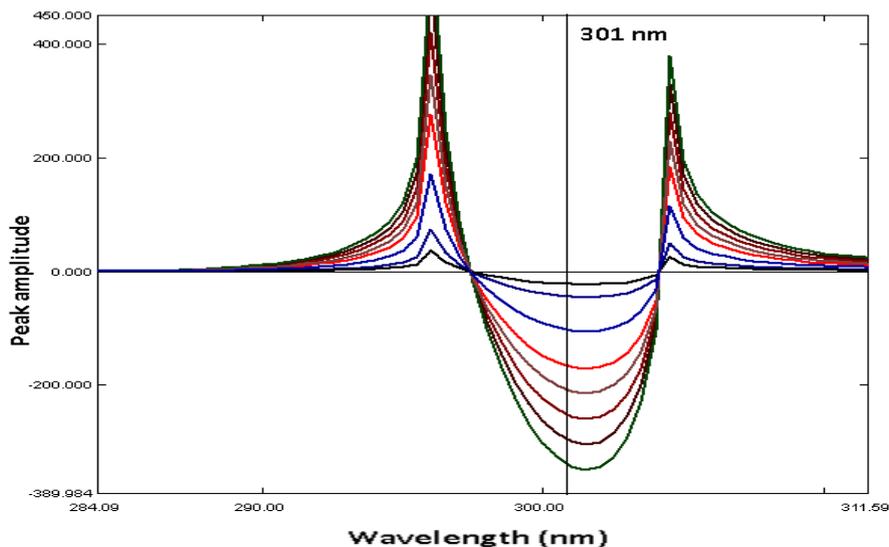
### 3.3. Ratio derivative spectrophotometric method [Abdallah, etal,2011]:

For CHZ, the absorption spectra of CHZ were divided by the absorption spectrum of DIC (10 µg/mL), as a divisor, to get the ratio spectra, as shown in **figure (6)**. The amplitudes of the first derivative of the ratio spectra at 290 nm were proportional to the concentrations of the drug without interference from DIC (divisor), as shown in **figure (10)**.

For DIC, the absorption spectra of DIC were divided by the absorption spectrum of CHZ (10 µg/mL), as a divisor, to get the ratio spectra, as shown in **figure (7)**. The amplitudes of the first derivative of the ratio spectra at 301 nm were proportional to the concentrations of the drug without interference from CHZ (divisor), as shown in **figure (11)**.

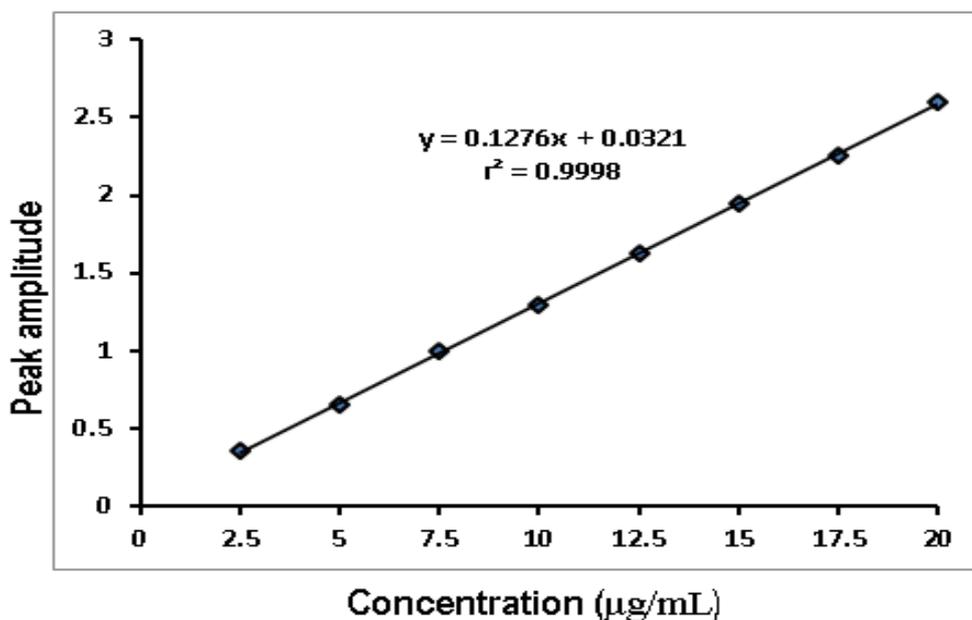


**Figure (10)** First derivative of the ratio spectra of chlorzoxazone at various concentrations (2.5 - 20 µg/mL) using 10 µg/mL of diclofenac potassium as a divisor.

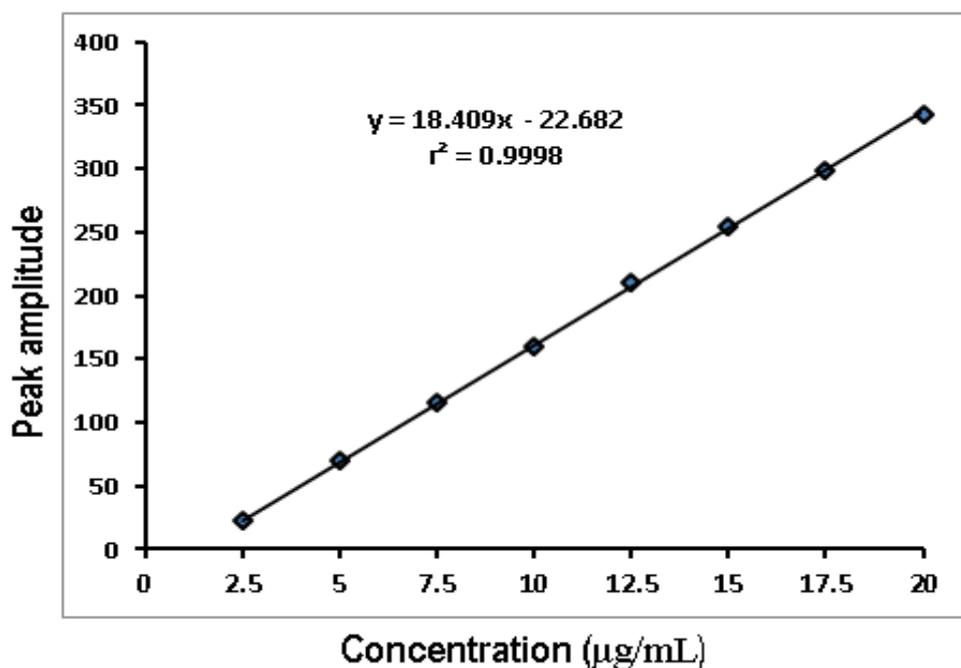


**Figure (11)** First derivative of the ratio spectra of diclofenac potassium at various concentrations (2.5 - 20 µg/mL) using 10 µg/mL of chlorzoxazone as a divisor.

Calibration curves for CHZ and DIC were constructed by plotting the peak amplitude values at the selected wavelengths for each drug against their corresponding concentrations as shown in **figures (12&13)**. CHZ and DIC obeyed Beer-Lambert's law in the concentration ranges of 2.5-20 µg/mL for both drugs with good correlation coefficients. Regression equations as follows:  $Y = 0.1276X + 0.0321$  for CHZ and  $Y = 18.409X - 22.682$  for DIC. Regression parameters are given in **table (1)**.



**Figure (12)** Calibration graph of chlorzoxazone by the proposed ratio derivative method.



**Figure (13) Calibration graph of diclofenac potassium**

**by the proposed ratio derivative method.**

#### **3.4. Constant center spectrophotometric method [Nassar MW,etal,2018]:**

By applying the theory of constant center method, the original spectra of CHZ(X) and DIC(Y) will be obtained. In constant center method, the absorption spectrum of the mixture (CHZ+ DIC) is scanned and divided by the absorption spectrum of a known concentration of one of the components as a divisor, respectively, and the ratio spectrum obtained represents (DIC/CHZ) + constant or CHZ/DIC +constant.

The selected divisors should compromise between minimal noise and maximum sensitivity. The divisor concentration 10 µg/ mL CHZ and DIC gave the best results regarding average recovery percent when used for the analysis of CHZ and DIC concentrations in mixtures, respectively.

Ratio difference at two selected wavelengths was applied to the ratio spectra of the cited drug.  $(DIC/CHZ)_1 - (DIC/CHZ)_2$  or  $(CHZ/DIC)_1 - (CHZ/DIC)_2$  where the interfering substance was canceled and subsequently show no interference. The only requirement for the selection of these two wavelengths is the contribution of the two components at these two selected wavelengths  $\lambda_1$  and  $\lambda_2$  where the ratio spectrum of the interfering component shows the same value (constant) whereas the component of interest shows a significant difference in these two ratio values at these two selected wavelengths with concentrations.

The two selected wavelengths are (246 nm and 284 nm) for CHZ and (280 nm and 230 nm) for DIC as shown in **figures (6 & 7)**.

For the determination of CHZ in the binary mixture, the zero order spectrum of the mixture was scanned and the ratio spectrum of the mixture was obtained using standard 10 µg/ mL CHZ\* as a divisor where practical amplitude at 246 nm were recorded (DIC/CHZ\* + CHZ/CHZ\*) for each laboratory prepared mixture, while the postulated amplitude value of (DIC/ CHZ\*) can be calculated using the equation representing the linear relationship between the ratio difference of ratio spectra at 246 nm and 284 nm (interfering component was canceled) versus the corresponding ratio amplitudes at 246 nm to calculate the constant value of CHZ via amplitude difference step using regression equation (1)

$$P_1 - P_2 = 0.8823P_1 + 0.0089 \quad (r^2=1) \quad (1)$$

where;  $P_1$ ,  $P_2$  are the ratio amplitudes at 246 nm and 284 nm using standard 10 µg/ mL CHZ\* as a divisor and DIC/ CHZ\* is the corresponding ratio amplitudes of the ratio spectra at 246 nm. The constant value was calculated by measuring the difference between the recorded amplitude and postulated amplitude at this wavelength.

$$C.V = (P \text{ recorded}) - (P \text{ postulated})$$

$$P (\text{CHZ/CHZ}^*) = P (\text{DIC/CHZ}^* + \text{CHZ/CHZ}^*) - P (\text{DIC/CHZ}^*)$$

where, C.V is the constant value, P recorded is the recorded amplitude of the ratio spectrum of the laboratory prepared mixture using standard 10 µg/ mL CHZ\* as a divisor at 246 nm and P postulated is the calculated amplitude using the specified regression equation (1).

Multiplying the obtained constant value representing CHZ/CHZ\* by the absorption spectrum of standard CHZ\* as a divisor. The obtained zero order absorption spectrum of CHZ **figure (14)** could be used for the determination of its concentration in the mixture by using the corresponding regression equation (  $Y = 0.0551X + 0.0154$ ) obtained by plotting the absorbance at its  $\lambda_{\text{max}}$  280 nm and its corresponding concentration as shown in **figure (16)** and the regression parameters established in **table (1)**.

Similarly DIC(Y) can be determined using standard 10 µg/ mL DIC\* as a divisor at (280 nm and 230 nm) versus 280 nm to calculate the constant value of DIC via the amplitude difference step using regression equation (2)

$$P_1 - P_2 = 0.0439P_1 + 0.0178 \quad (r^2=0.9997) \quad (2)$$

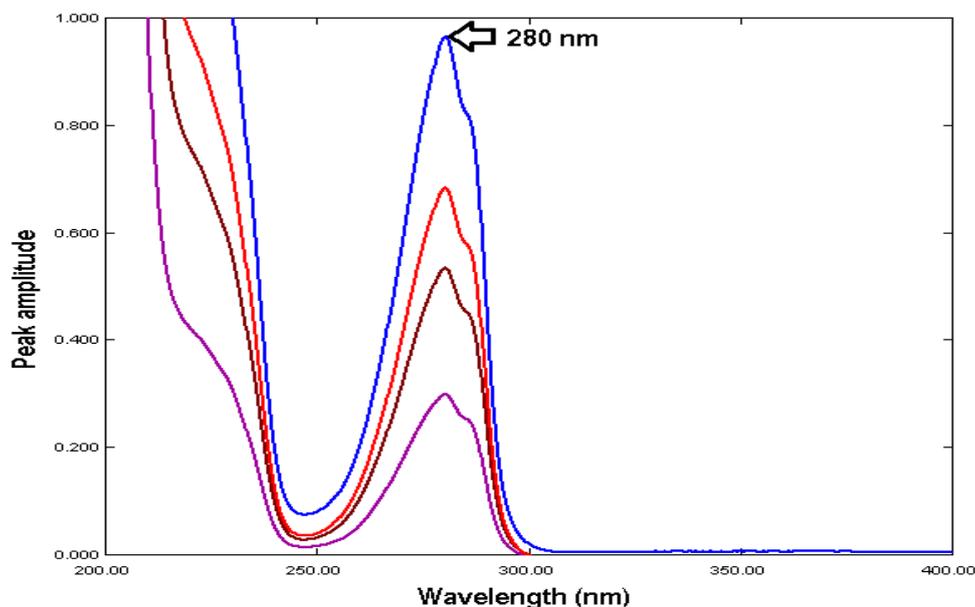
where;  $P_1$ ,  $P_2$  are the ratio amplitudes at 280 nm and 230 nm using standard 10 µg/ mL DIC\* as a divisor and CHZ/ DIC\* is the corresponding ratio amplitudes of the ratio spectra at 280 nm. The constant value was calculated by measuring the difference between the recorded amplitude and postulated amplitude at this wavelength.

$$C.V = (P \text{ recorded}) - (P \text{ postulated})$$

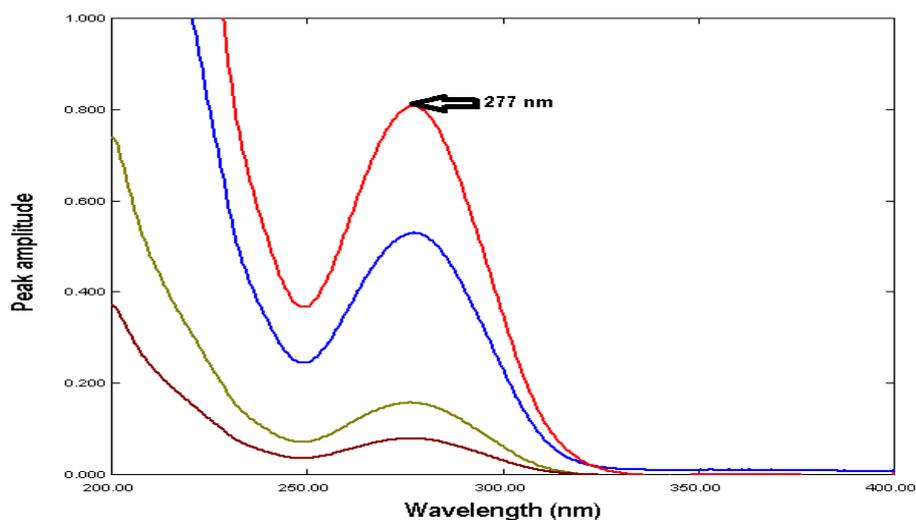
$$P (\text{DIC}/\text{DIC}^*) = P (\text{CHZ}/\text{DIC}^* + \text{DIC}/\text{DIC}^*) - P (\text{CHZ}/\text{DIC}^*)$$

where, C.V is the constant value, P recorded is the recorded amplitude of the ratio spectrum of the laboratory prepared mixture using standard 10  $\mu\text{g}/\text{mL}$  DIC\* as a divisor at 280 nm and P postulated is the calculated amplitude using the specified regression equation (2).

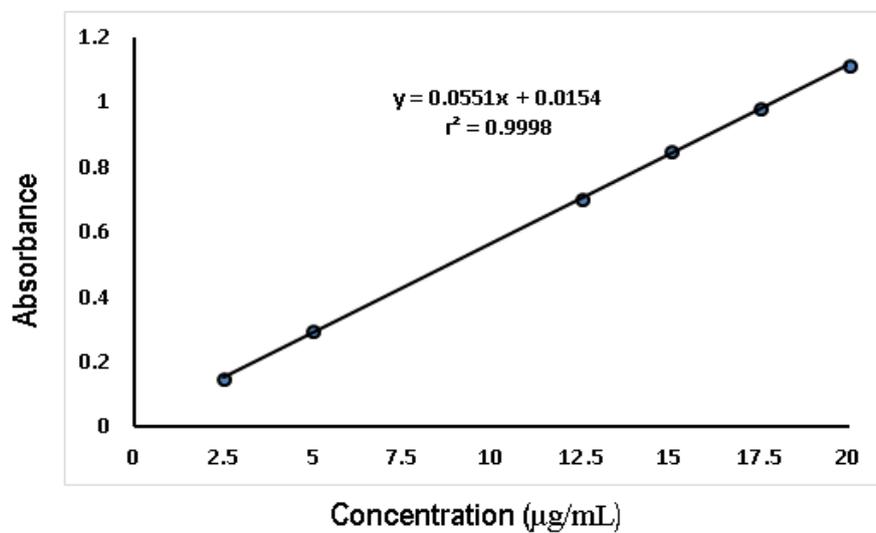
Multiplying the obtained constant value representing DIC/DIC\* by the absorption spectrum of standard DIC\* as a divisor. The obtained zero order absorption spectrum of DIC **figure (15)** could be used for the determination of its concentration in the mixture by using the corresponding regression equation ( $Y = 0.0591X - 0.0713$ ) obtained by plotting the absorbance at its  $\lambda_{\text{max}}$  277 nm and its corresponding concentration as shown in **figure (17)** and the regression parameters established in **table (1)**.



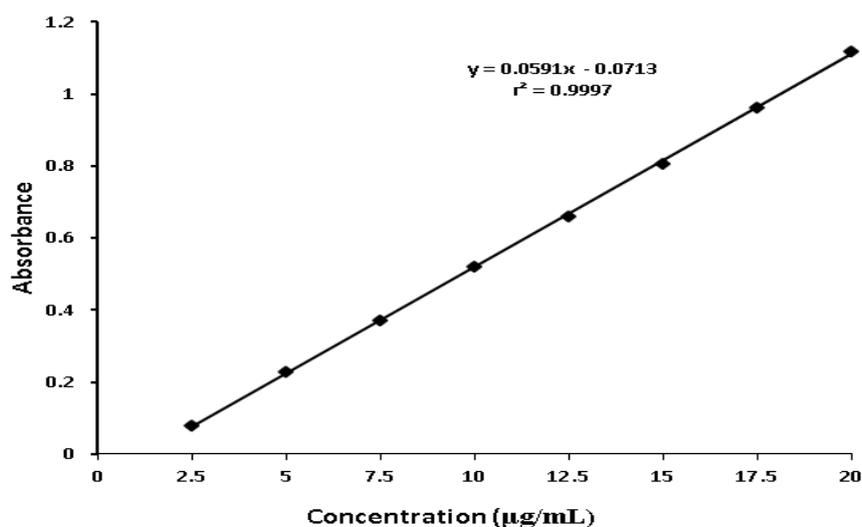
**Figure (14) The final spectra of chlorzoxazone at various concentrations (5,10,12.5,17.5  $\mu\text{g}/\text{mL}$ ) after multiplication by the spectrum of 10  $\mu\text{g}/\text{mL}$  of chlorzoxazone.**



**Figure (15) The final spectra of diclofenac potassium at various concentrations (2.5,5,10,15 µg/mL) after multiplication by the spectrum of 10 µg/mL of diclofenac potassium.**



**Figure (16) Calibration graph of chlorzoxazone by the proposed constant center method.**



**Figure (17) Calibration graph of diclofenac potassium by the proposed constant center method.**

**Optimization of experimental conditions for the last three ratio manipulating spectra spectrophotometric methods:**

Careful choice of the divisor concentration was of great importance, so different concentrations of each drug were tried as a divisor (4, 6, 8, 10 and 14 µg/ mL); the best one was 10 µg/ mL, as it gave better results in accordance with selectivity. The statistical parameters of the regression equations are shown in **table (1)** & **table (2)**. The percentage recoveries suggested good accuracy of the proposed methods.

The obtained results by the suggested methods were statistically compared with those obtained by the reported method [Patel SA,etal,2013], showing no significant difference with respect to accuracy and precision at  $p = 0.05$ .

The proposed methods were successfully applied for the analysis of CHZ and DIC in their pharmaceutical solid dosage form (capsule) and in their laboratory prepared mixtures, **table (3)** & **table (4)**. Statistical comparison between the results obtained by the proposed methods and those obtained by the reported method showed no significant differences as given in **table (3)**. In order to compare the ability of the proposed methods for the determination of CHZ and DIC in their pharmaceutical solid dosage form (capsule), the results obtained by applying the proposed methods were subjected to statistical analysis using the one way ANOVA test, there was no significant difference between all of the proposed methods, **table (5)**.

Table (1): Regression parameters for simultaneous determination of the studied drugs by the proposed spectrophotometric methods:

Method	Drug	Wavelength (nm)	Linearity range ( $\mu\text{g/mL}$ )	Slope	Intercept	Coefficient of determination ( $r^2$ )	LOD ( $\mu\text{g/mL}$ )	LOQ ( $\mu\text{g/mL}$ )
Dual wavelength	CHZ	259-294nm	2.5-20	0.0027	0.0094	0.9997	0.308	0.458
	DIC	294-242nm	2.5-20	0.003	0.0077	0.9997	0.932	1.388
Ratio difference	CHZ	280-230nm	2.5-20	0.0447	0.0072	0.9998	0.307	0.929
	DIC	246-284nm	2.5-20	0.894	-1.245	0.9997	0.373	1.123
Ratio derivative	CHZ	290	2.5-20	0.128	0.032	0.9998	0.287	0.871
	DIC	301	2.5-20	18.409	-22.682	0.9998	0.301	0.911
Constant center	CHZ	280	2.5-20	0.0551	0.0154	0.9998	0.221	0.521
	DIC	277	2.5-20	0.0591	-0.0713	0.9997	0.331	0.825

Table (2): validation data for simultaneous determination of the studied drugs by the proposed spectrophotometric methods:

Method	Drug	Accuracy (%R)* mean $\pm$ SD	precision repeatability	Intermediate precision
Dual wavelength	CHZ	98.99 $\pm$ 1.312	1.523	1.899
	DIC	101.23 $\pm$ 1.687	1.122	1.754
Ratio difference	CHZ	99.94 $\pm$ 1.064	0.713	0.734
	DIC	101.53 $\pm$ 1.392	0.528	0.832
Ratio derivative	CHZ	99.73 $\pm$ 1.211	1.151	1.715
	DIC	100.26 $\pm$ 1.600	1.232	1.322
Constant center	CHZ	101.22 $\pm$ 0.925	0.945	0.875
	DIC	98.59 $\pm$ 1.392	0.854	1.325

\* Values for 3 determinations of 3 different concentrations

Table (3): Determination of the studied drugs in the pharmaceutical preparation by the proposed methods and statistical comparison with reported Q-absorbance ratio method :

Method	Drug	Declophen plus <sup>®</sup> capsules <sup>a</sup> (B.N. 210102)	Standard addition <sup>b</sup>	F-test <sup>c</sup> (6.388)	Student's t-test <sup>c</sup> (2.306)
Dual wavelength	CHZ	100.59±1.304	101.22±1.235	0.680	0.869
	DIC	99.41±1.696	101.52±1.321	1.467	1.828
Ratio difference	CHZ	101.05±1.053	100.521±1.024	0.447	1.475
	DIC	100.80±1.630	99.56±1.058	1.393	0.415
Ratio derivative	CHZ	100.94±1.200	101.22±0.941	0.579	1.289
	DIC	99.37±1.610	98.25±0.854	1.321	1.933
Constant center	CHZ	100.85±0.917	99.52±0.523	0.338	1.291
	DIC	101.04±0.705	100.21±0.984	0.262	0.232
Reported method(Patel SA,etal,2013)	CHZ	99.79±1.595			
	DIC	101.20±1.376			

a Average of five determinations.

b Average of four determinations.

c The values in the parenthesis are the corresponding theoretical values at p= 0.05.

Table (4): Determination of the studied drugs in laboratory prepared mixtures by the proposed spectrophotometric methods:

Method	Chlorzoxazone taken (µg/mL)	Diclofenac taken (µg/mL)	Chlorzoxazone found (µg/mL)	Diclofenac found (µg/mL)	% Recovery of Chlorzoxazone	% Recovery of Diclofenac
Dual wavelength	10	10	10.11	9.88	101.10	98.80
	10	5	9.98	4.97	99.80	99.40
	12.5*	2.5*	12.40	2.53	99.20	101.20
	17.5	2.5	17.23	2.54	98.46	101.60
	5	15	5.05	15.23	101.00	101.53
	Mean ± %RSD					99.91±1.145
Ratio derivative	10	10	9.98	10.10	99.80	101.00
	10	5	9.98	5.05	99.80	101.00
	12.5*	2.5*	12.30	2.52	98.40	100.80
	17.5	2.5	17.30	2.54	98.86	101.60
	5	15	4.98	15.30	99.60	102.00
	Mean ± %RSD					99.29±0.636
Ratio derivative	10	10	10.05	10.20	100.50	102.00
	10	5	10.12	4.94	101.20	98.80
	12.5*	2.5*	12.40	2.53	99.20	101.20
	17.5	2.5	17.20	2.55	98.29	102.00
	5	15	5.05	14.95	101.00	99.67
	Mean ± %RSD					100.04±1.251
Constant center	10	10	10.15	10.12	101.50	101.20
	10	5	10.19	5.06	101.90	101.20
	12.5*	2.5*	12.52	2.55	100.16	102.00
	17.5	2.5	17.52	2.54	100.11	101.60
	5	15	5.03	15.21	100.60	101.40
	Mean ± %RSD					100.85±0.800

\*Ratio present in declophen plus<sup>®</sup> capsules.

Table (5): One-way ANOVA test for the different proposed spectrophotometric methods used for the determination of the studied drugs in declophen plus<sup>®</sup> capsules:

Source of variation	Sum of squares	Degree of freedom	Mean of squares	F*
Between groups	22.343	9	2.483	1.360
Within groups	73.023	40	1.826	(2.124)

\* The value in parenthesis is the critical value of "F" at (P = 0.05).

### Conclusion

In this work smart and simple developed spectrophotometric methods were applied for the analysis of CHZ and DIC in their binary mixture. The developed methods do not need sophisticated instruments or any prior separation steps and so it can be used as alternative methods to liquid chromatographic methods in laboratories lacking the required facilities for these techniques for the analysis of any binary mixture without any limitation or specified requirements except that the two spectra of the proposed drugs should be contributing at the chosen wavelengths.

The proposed methods could be easily applied in quality control laboratories as they are having similar accuracy and precision compared to the reported method.

Dual wavelength method utilize zero order spectra without any division as in ratio difference method or division then derivatization as in ratio derivative method or division then multiplication as in constant center method so, it is the best method as it is not need several manipulation steps.

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### Conflict of interest

The author declares that there is no conflict of interest.

### REFERENCES

- Abdallah OM, Badawey AM. (2011) Derivative- ratio spectrophotometric, chemometric and HPLC validated methods for simultaneous determination of amlodipine and atorvastatin in combined dosage form. *Int J Ind Chem.*;2(2):78-85 .

- Ahmed HM, Elshamy YS, Talaat W, Labib HF, Belal TS. (2020)** *Simultaneous analysis of chlorzoxazone, diclofenac sodium and tramadol hydrochloride in presence of three potential impurities using validated HPLC-DAD and HPTLC methods. Microchem J.; 153*
- Badgujar MA, Pingale SG, Mangaonkar KV. (2011)** *Simultaneous determination of paracetamol, chlorzoxazone and diclofenac sodium in tablet dosage form by high performance liquid chromatography. E J Chem.;8(3):1206-1211.*
- British pharmacopoeia 2013. Vol. 2. London,UK: The stationery office;2012.**
- Fernandes N, Nimdeo MS, Choudhar VP, Kulkarni RR, Pande VV, Nikalje AG.(2008)** *Dual wavelength and simultaneous equation spectrophotometric methods for estimation of atenolol and indapamide in their combined dosage form. Int J Chem Sci.;6(1):29-35.*
- Hegazy MA, Elshahed MS, Toubar SS , Marwa I. Helmy ML.(2018)** *Efficient processing of single and multiple spectral variables for resolution and quantitation of paracetamol, chlorzoxazone and diclofenac. J Adv Pharm Res.; 2(4):269-282.*
- Krutika P, Kumar VS, SG V.(2021)** *Stability indicating assay method development and validation of simultaneous estimation of chlorzoxazone, diclofenac sodium and paracetamol in bulk drug and tablet by RP-HPLC. Res J Pharm Tech.; 14(9)*
- Lotfy HM, Saleh SS, Hassan NY, Elgizawy SM.(2012)** *A Comparative study of the novel ratio difference method versus conventional spectrophotometric techniques for the analysis of binary mixture with overlapped spectra. Am J Anal Chem.; 3:761-769.*
- Nassar MW, Attia KA, Abouserie AA, Said RA, AbdelKareem RF.(2018)** *Comparative study on four UV spectrophotometric methods manipulating ratio spectra for the determination of metoclopramide monohydrochloride monohydrate in presence of its acidic degradate. J Anal Pharm Res.;7(3):344-355.*
- Panchumarthy R, Garikapati DR, Akaula A, Puttagunta SB.(2013)** *A Validated RP HPLC method for simultaneous estimation of paracetamol, diclofenac sodium and chlorzoxazone in combination tablet dosage form. J Chem Pharm Sci.;6(3):181-188.*
- Patel RA, Joshi SH.(2014)** *Development and validation of spectrophotometric methods for simultaneous estimation of diclofenac sodium and serratiopeptidase in pharmaceutical dosage form. World J Pharm Pharm Sci.;3:1279-1291.*

**Patel SA, Prajapati KM.(2013)** *Spectrophotometric estimation of chlorzoxazone and diclofenac sodium in synthetic mixture by Q-absorbance ratio method. Int J Chem Tech Res.;*5(4):2034-2040.

**Sakinala P, Vahika H, Santhosh K, Attinti S.(2020)** *Development and validation of stability indicating assay for simultaneous determination of pentaprazole, diclofenac, chloroxazone in pharmaceutical dosage form by using RP-HPLC. Inter J Pharm Sci Res.;* **11(4): 1757-1767.**

**United States Pharmacopeia 34 and National formulary 29. Rockville (MD): United State Pharmacopeia convention;**2011.

## دراسة مقارنة بين أربع طرق طيفية لتحديد المزيج الثنائي لكلورزوكسازون وديكلوفيناك البوتاسيوم في وقت واحد

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تم تطوير أربع طرق بسيطة وسريعة ودقيقة وقابلة للتكرار وغير معقدة لقياس الطيف الضوئي والتحقق من صحتها من أجل التحديد المتزامن لكلورزوكسازون وديكلوفيناك البوتاسيوم دون فصل أولي في شكل مسحوق نقي وفي الكبسولات التجارية. الطريقة أ هي طريقة قياس طيفي مزدوج الطول الموجي حيث كانت الأطوال الموجية المختارة لتقدير الكلورزوكسازون ٢٥٩ نانومتر و ٢٩٤ نانومتر ، بينما الأطوال الموجية المختارة لتقدير ديكلوفيناك البوتاسيوم كانت ٢٩٤ نانومتر و ٢٤٢ نانومتر. بينما الطريقة ب هي طريقة قياس طيفي فرق النسبة حيث كانت الأطوال الموجية المختارة لتقدير الكلورزوكسازون ٢٨٠ نانومتر و ٢٣٠ نانومتر ، بينما كانت الأطوال الموجية المختارة لتقدير ديكلوفيناك البوتاسيوم ٢٤٦ نانومتر و ٢٨٤ نانومتر. بينما الطريقة ج هي المشتق الأول لأطياف النسبة المقاسة عند ٢٩٠ نانومتر و ٣٠١ نانومتر لكلورزوكسازون وديكلوفيناك البوتاسيوم على التوالي ، بينما الطريقة د هي طريقة قياس الطيف الضوئي الثابت عند ٢٨٠ نانومتر و ٢٧٧ نانومتر لكلورزوكسازون وديكلوفيناك البوتاسيوم على التوالي ، أظهر تحليل الانحدار لمخططات بير-لامبرت ارتباطاً جيداً في نطاق التركيز ٢.٥ - ٢٠ ميكروغرام / مل لكلا العقارين مع كشف أقل كمية مقدارها 0.308 ميكروغرام / مل و ٠.٩٣٢ ميكروغرام / مل ، قياس أقل كمية مقدارها 0.458 ميكروغرام / مل و ١.٣٨٨ ميكروغرام / مل ، انحراف معياري نسبي مقداره 1.325 و ١.٦٦٦ و دقة مقدارها ٩٨.٩٩ و ١٠١.٢٣ لكلورزوكسازون وديكلوفيناك بوتاسيوم ، على التوالي بالطريقة أ.

علاوة على ذلك ، كشف أقل كمية مقدارها 0.307 ميكروغرام / مل و ٠.٣٧٣ ميكروغرام / مل ، قياس أقل كمية مقدارها 0.929 ميكروغرام / مل و ١.١٢٣ ميكروغرام / مل ، انحراف معياري مقداره 1.065 و ١.٣٧١ و دقة مقدارها ٩٩.٩٤ و ١٠١.٥٣ لكلورزوكسازون وديكلوفيناك بوتاسيوم ، على التوالي في الطريقة B. علاوة على ذلك ، كشف أقل كمية مقدارها 0.287 ميكروغرام / مل و ٠.٣٠١ ميكروغرام / مل ، قياس أقل كمية مقدارها 0.871 ميكروغرام / مل و ٠.٩١١ ميكروغرام / مل ، انحراف معياري مقداره 1.214 و ١.٥٩٦ و دقة مقدارها ٩٩.٧٣ و ١٠٠.٢٦ لكلورزوكسازون وديكلوفيناك البوتاسيوم ، على التوالي في الطريقة C. علاوة على ذلك ، كشف أقل كمية مقدارها 0.221 ميكروغرام / مل و ٠.٣٣١ ميكروغرام / مل ، قياس أقل كمية مقدارها 0.521 ميكروغرام / مل و ٠.٨٢٥ ميكروغرام / مل ، انحراف معياري مقداره 0.914 و ١.٤١٢ و دقة مقدارها ٩٨.٥٩ و ١٠١.٢٢ لكلورزوكسازون وديكلوفيناك البوتاسيوم ، على التوالي في الطريقة د.

تم التحقق من صحة الطرق المقترحة في الامتثال لإرشادات المجلس الدولي للمواءمة وتم تطبيقها بنجاح لتقدير كلورزوكسازون وديكلوفيناك البوتاسيوم في خليطهم المعد في المعمل والكبسولات التجارية.

**الكلمات الرئيسية :** الطول الموجي المزدوج. فرق النسبة. مشتق نسبة مركز ثابت. كلورزوكسازون. ديكلوفيناك البوتاسيوم. خليط ثنائي.