ZANCO Journal of Pure and Applied Sciences
The official scientific journal of Salahaddin University-Erbil
https://zancojournals.su.edu.krd/index.php/JPAS

ISSN (print): 2218-0230**, ISSN (online):** 2412-3986**, DOI: http://dx.doi.org/10.21271/zjpas**

RESEARCH PAPER

Q-ABSORBANCE RATIO, DERIVATIVE SPECTROPHOTOMETRIC AND H-POINT STANDARD ADDITION METHODS FOR SIMULTANEOUS DETERMINATION OF PRIDINOL MESYLATE, AND DICLOFENAC SODIUM IN PHARMACEUTICAL FORMULATION.

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A B S T R A C T:

Three methods for simultaneous estimation of diclofenac sodium and pridinol mesylate have been developed. First method is the application of the Q–analysis (absorbance ratio) method, the wavelengths selected were 265nm (isosbestic point) and 285nm (λ_{max} of diclofenac sodium) with 258nm (λ_{max} of pridinol mesylate). The linearity ranges for diclofenac sodium and pridinol mesylate were 8-64 µg/ml and 14-56 µg/ml, respectively. The second technique is based on the second derivative spectrophotometric method at zero crossing wavelengths. The linearity ranges for diclofenac sodium and pridinol mesylate were (4.0-40.0) µg/ml and (4.0-30.0) µg/ml, respectively. The third method is the H-Point Standard addition method (HPSAM) depending upon the zero and second-order derivative signals for diclofenac sodium and pridinol mesylate were applied pairs of wavelengths, 250.37and 266.34 nm; with 224.18 and 235.10 nm, respectively.

The accuracy of the methods was assessed by recovery studies and was found to be 100.01 ± 0.63 and 100.64 ± 0.36 for the q-absorbance ratio method, 100.78 ±0.221 and 101.98 ±0.11 for the second derivative zero-crossing method, respectively. While for H-Point Standard addition method are 98.0±1.71, and 100.4±0.545 for zero-order HPSAM , 99.25 ±0.753, and 100.12 ± 0.35 for second derivative HPSAM for diclofenac sodium and pridinol mesylate respectively

Methods were confirmed according to the ICH guidelines; accuracy, precision, and repeatability were found to be within acceptable limits with no interferences. Finally, statistical comparisons between the proposed methods and the reported methods concerning to accuracy and precision show that no significant difference was found using Student's t-test, F-test, and one-way ANOVA.

KEY WORDS: Q-analysis, Derivative spectrophotometry, HPSAM, Diclofenac sodium, and Pridinol mesylate.
DOI: http://dx.doi.org/10.21271/ZJPAS.33.4.5
ZJPAS (2021), 33(4); 43-60 .

1.INTRODUCTION :

Pridinol mesylate (PriM) (Figure 1) is a vital anticholinergic drug, with beneficial muscle relaxant properties (Salazar-Rojas et al., 2019) and working as a myotonolytic and spasmolytic agent in antistress cure and for the treatment action of Parkinson's disease (Wongrakpanich et al., 2018, Vignaduzzo et al., 2011),

Pridinol mesylate (PriM) is most commonly formulated in link with non-steroidal anti-inflammatory drugs, containing diclofenac, meloxicam and piroxicam, for the usage and treatment of muscular contractures and low back pain (Abdel Shaheed et al., 2017) **.** Diclofenac sodium (DicNa) (2-(2,6-dichloroanilino)Phenylacetate) (Figure 2) is a non-steroidal anti-inflammatory drug (Lins et al., 2020) supported for use in painful and inflammatory rheumatic and definite non-rheumatic conditions (Moore et al., 2019, Lins et

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Article History:
Received: 23/03/2021
Accepted: 31/05/2021
Published: 18/08 /2021

al., 2020), and antiarthritic source. It is suggested to decrease menstrual pain, dysmenorrhea, etc. (Anggraini and Ekawati, 2020) . Yearly intake of diclofenac is 940 tons universally with a mentioned dose of 100 mg/day (Tiwari et al., 2015).

Recently, there is a new combination consisting of DicNa, or ternary mixture combined above with cyanocobalamin (vitamin B12) have been released in the markets (Kleemann et al., 2014).

Several analytical methods have been reported for estimation of these ingredients PriM, and DicNa, separately or in combination with other compounds using HPLC(Anggraini and Ekawati, 2020, Brezovska et al., 2013, Gunji et al., 2012, Vignaduzzo et al., 2010), LC-MS (Yang et al., 2019, Chethana et al., 2012, Michopoulos et al., 2015) , UV-visible spectrophotometry (Mane et al., 2019, Darweesh et al., 2018, Dikran and Mahmood, 2017) Electrochemical methods (Kimuam et al., 2020, Afkhami et al., 2016, Robinson et al., 2014).

In the present work, Q-absorbance ratio method, zero-crossing second derivative spectrophotometric method and applied H-point standard addition method (HPSAM), second-order derivative spectra(2D-HPSAM), and the zero order derivative spectra (ZO-HPSAM), for simultaneous estimation of DicNa and PriM in bulk and pharmaceutical formulation.

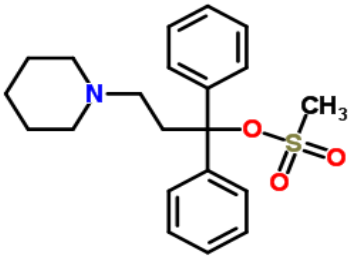

Figure (1): Chemical structure of pridinol mesylate (PriM).

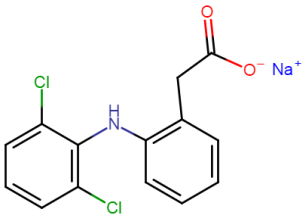

Figure (2): Chemical structure of diclofenac sodium (DicNa)

2. MATERIAL AND METHODS

2.1 Apparatus

A dual UV-Visible Spectrophotometer (Perkin Elmer Lambda 35.) was connected to identical 1.0 cm quartz cells to a computer (Wind XP), and the software used SHIMADZU UV probe data system software (2.34 ink.), using the following parameters: scan speed (240), slit width (02), delta lambda (10); Scaling factor (25), mode 2D (second derivative); The wavelength range (200-3450nm) and the digital calipers within the operating instructions (100-300mm) were used to measure height of the peaks.

2.2 Stock solutions and working solutions

Standard stock solutions of 100 µg mL^{-1} of DicNa and PriM (purchased in Glentham Life Sciences Ltd. (UK)) for binary mixture analysis were prepared by precisely weighing 100 mg of DicNa and PriM, separately into two separate 100mL volumetric flasks and then complete it to the mark with ethanol, multisolvent® HPLC grade (TDS, ET00152500) Scharlau, S.L. (Spain).

Working solutions were prepared from their own standard stock solutions diluting by ethanol into 100mL volumetric flasks containing (1.0–40.0, and 1.0–30.0, µgmL−1, DicNa and PriM), and their laboratory-prepared mixtures were prepared from their own working standard solutions in different proportions both standard stock solutions and working solutions were prepared daily.

2.3 Pharmaceutical formulation

Ten tablets of Dioxaflex® Plus (purchased from Gramón Bagó de Uruguay S.A.) weighed, powdered and mixed in a mortar for the pharmaceutical formulation solutions. 10mg of powdered drug was dissolved in 100 mL of ethanol. The solution was filtered by syringe filter 0.4µm before preparing different concentrations by serial dilution. Ethanol, multisolvent® HPLC grade (TDS, ET00152500) Scharlau, S.L. (Spain) was used as a solvent.

2.4 Condition and absorption for zero order spectra

The normal UV absorption spectra of (DicNa, and PriM) and their mixture were recorded against absolute ethanol as a solvent blank. (Figure 3) shows absorption spectrum of 20 µg/ml of DicNa solution with lambda max at 285 nm, absorption spectrum of 15 µg/mL of PriM solution with a maximum peak at 258 nm, and isosbestic point were obtained at 265 nm. The overlap spectra of DicNa and PriM at two different concentration ranges were recorded. In the absorption spectrum of the mixture shows an overlap spectrum; therefore, the simultaneous determination is very difficult due to spectral interference.

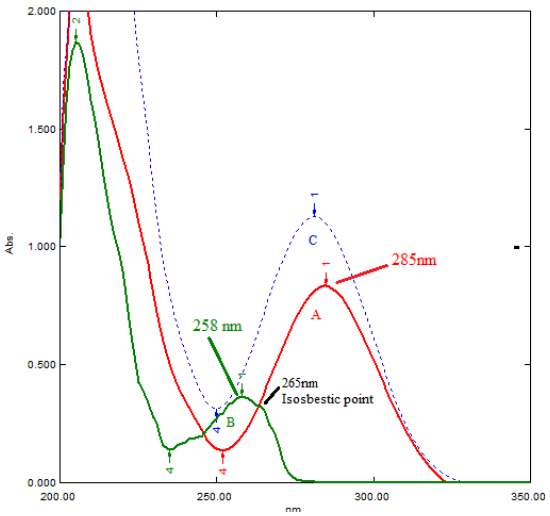

Figure (3): Zero-order spectra of (a) 20 µg/mL diclofenac sodium (DicNa), (b) 15 µg/mL pridinol mesylate (PriM), and (c) mixture of DicNa and PriM against absolute ethanol as a blank.

2.5 Q-absorbance ratio method

This method is valid to the drugs that obey Beer's law at all wavelengths and the ratio of absorbance at any two wavelengths is a constant value, (Khamar and Patel, 2012) . At wavelengths Figure 3 shows two pair wavelengths, 265nm as isosbestic point, 285nm (λmax of DicNa), and 258nm (λmax of PriM) and selected for the formation of the Q-absorbance ratio equation. The absorptivity coefficients of each compound at both wavelengths were determined. The concentration of the different components, CX (PriM)and CY (DicNa) can be planned and calculated by using the next following equations (Guideline, 2005, Patil et al., 2016).

CX = (QM-QY/QX-QY) x (A1/ax1) ……. [1]

CY = (QM-QX/QY-QX) x (A2/ay1) ……. [2]

Where, A1and A2 are absorbance of the mixture at 258 nm and 265 nm; ax1 and ay1 are absorptivity of DicNa and PriM at 258 nm; ax2 and ay2 are absorptivity of DicNa and PriM respectively at 265 nm; QM= A2 / A1, QX = ax2 / ax1 and QY = ay2 / ay1, wherever QM= (absorption ratio of isosbestic point to PriM) QX= absorptivity ratio at 258nm , and QY = absorptivity ratio at 265nm.

2.6 Condition and chosen second order derivative spectra

A set of 25 mL volumetric flasks, containing constant 20 µg/ml of DicNa and different amounts of PriM (1–30) µg/ml, while another set of 25 mL volumetric flasks, contain different amounts (1-40) µg/ml of DicNa, and PriM kept constant(15 µg/ml). The solutions were diluted with ethanol. The spectra of solutions were recorded against absolute ethanol as a solvent blank in the range of 200 –350 nm.

Considering and pending all the orders of derivative spectra of (DicNa, and PriM) from first to the fourth-order derivative, the graphical study revealed that second order of the derivative spectra as shown in (Figure 4) , was found to be simple and provide the best results for simultaneous determinations of (DicNa, and PriM) basing upon zero-crossing technique. This approach of quantitative determination allows the simultaneous guarantee of both analytes in a sample (Patel et al., 2010).

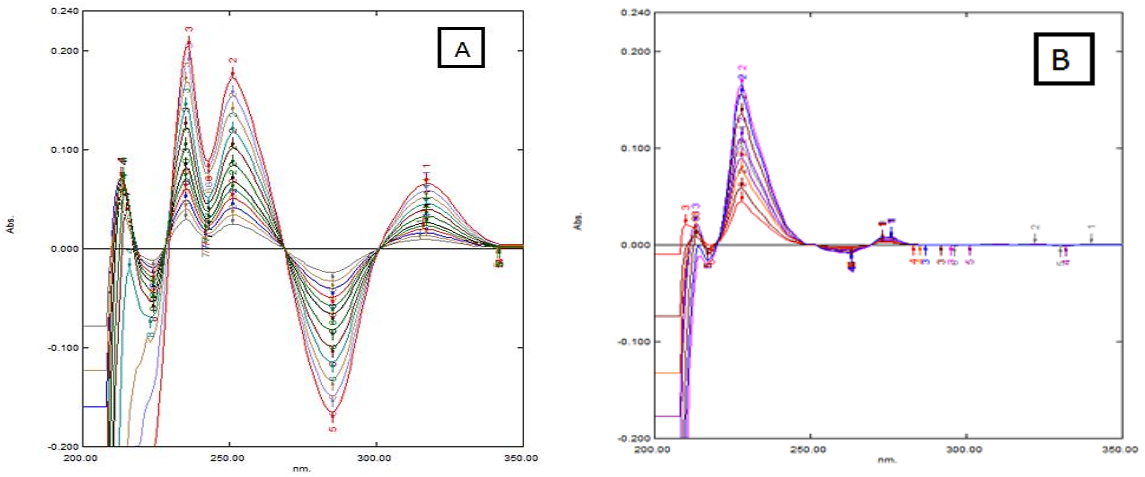

Figure (4): The overlain spectra of the second derivative of (a) 4-40 µg/mL pure DicNa and (b) 4-30 µg/mL pure PriM against absolute ethanol as a blank

2.7 Wavelength selection for HPSAM

Campins-Falco and et al. proposed an alteration and reform of the standard additions method technique known as the H-point standard additions method (HPSAM) in order to find an unbiased analyte concentration when both analyte and interferent are present in a sample. It also allowance the determination of the interferent concentration when it is known to be present (Świt et al., 2018) (Andrés et al., 1995, Reig, 1990).

To choose the suitable wavelength pair for applying of determination of DicNa and PriM in binary mixture by ZO-HPSAM and 2D-HPSAM, the following principles were applied:

1. At a selection of specific wavelengths the analyte signals necessity be linear to their concentration, and the interferent signal must remain unaffected and unchanged while varying the analyte concentration.
2. The analytical signal found from a mixture containing both analyte and the interferent should be equivalent to the sum of the specific individual or separate signals of two components.
3. The difference in the slopes of two straight lines recorded at the two selected wavelengths (λ1 and λ 2) need to be as large as possible in order to obtain decent and good sensitivity and accuracy (Andrés et al., 1995, Reig, 1990).

2.8 Condition and absorption spectra for HPSAM

The absorption spectrum of 8 µg/mL PriM, 8 µg/mL DicNa, and their mixture of PriM and DicNa were plotted against ethanol as a solvent blank on a 200 to 350 nm scale. As can be seen in (Figure 5), DicNa and PriM can be considered as an analyte and interference, respectively. In this state, and in view of these principles, the higher the slope value, the smaller the analyte concentration error, numerous pairs of wavelengths with the similar PriM absorbance were checked, the best pair of (250.37 and 266.34) nm higher slope values (Mλ1 = 0.0252, Mλ2 = 0.0114) were selected for zero-order spectra(ZO-HPSAM). Depending upon the mentioned principles the method developed by taking the second derivative mode (2D-HPSAM) instead of the normal one (Figure 6), the higher the slope value (M λ1 = 0.0581, M λ2 = 0.0294) with the best pair of (224.18 and 235.10) nm were selected.

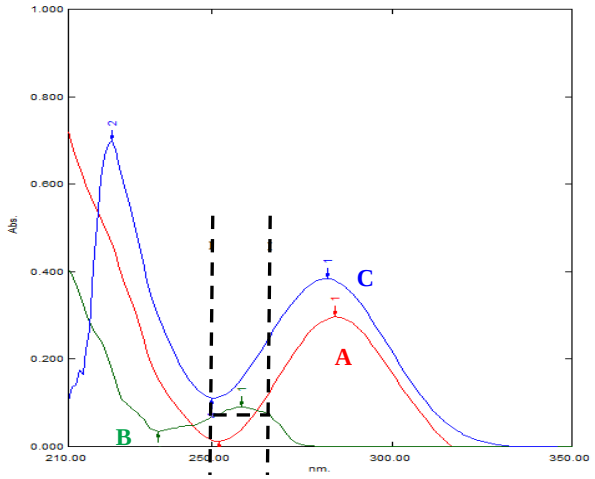

Figure (5): Overlain absorption spectra for (ZO-HPSAM) of (A) DicNa being the analyte, (B)PriM being the interference and (C) their mixture in absolute ethanol

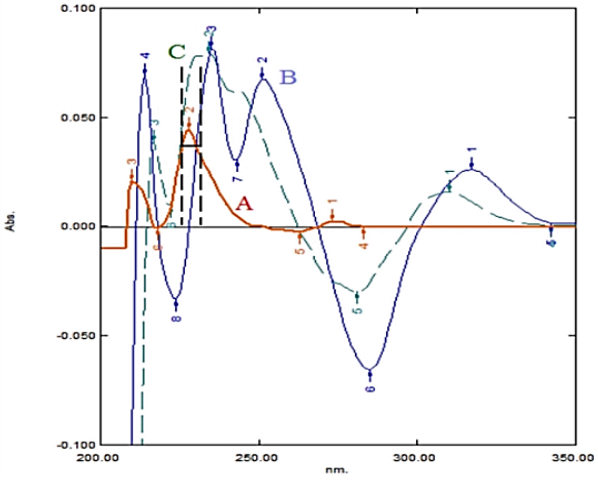

Figure (6): Overlain absorption spectra for (2D-HPSAM) of (A) DicNa being the analyte, (B)PriM being the interference and (C) their mixture in absolute ethanol

3. RESULT AND DISCUSSION

3.1 Second derivative mode study

Extremely overlapped spectra can be resolute by using derivative spectrophotometry (Kus et al., 1996). Derivative spectra can be used to resolve overlapping bands in qualitative and quantitative analysis and improve differences among spectra (O'haver and Green, 1976).

From these points of view zero-cross technique as shown in Figure 7, was used for the determination of PriM at the presence of DicNa in the solution, as follows:

Firstly at 228.2 nm DicNa undergoes zero absorption, but PriM has a positive peak. Undoubtedly at 251.69 nm and 283.98 PriM undergoes zero absorption at these points, furthermore, DicNa has a peak at positive and negative valleys respectively.Likewise, at 268.67 nm point, DicNa undergoes zero absorption, whereas PriM has a peak of negative valleys.Finally, any peak that exist after 300nm belongs to DicNa, while PriM does not have any peaks,therefore, in the present effort work, graphically studying method techniques (peak-to-baseline, height measuring, and zero-crossing) were used to contract with derivative spectra to achieve the simultaneous measurements at these points.

Figure 8 shows sets of 2nd order spectra of mixtures consist of fixed amounts of PriM (15.0 µg/mL) and in the presence of different concentrations (4-40 µg/mL) DicNa respectively.

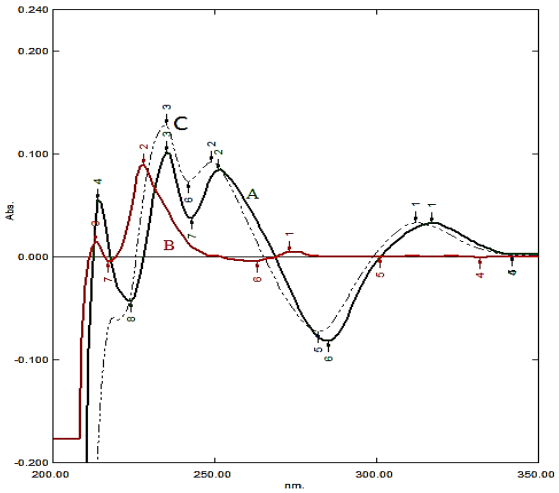

Figure (7): Second derivative spectra of: (A) 20 µg/mL DicNa,(B) 15.0 µg/mL PriM, and their mixture (C)

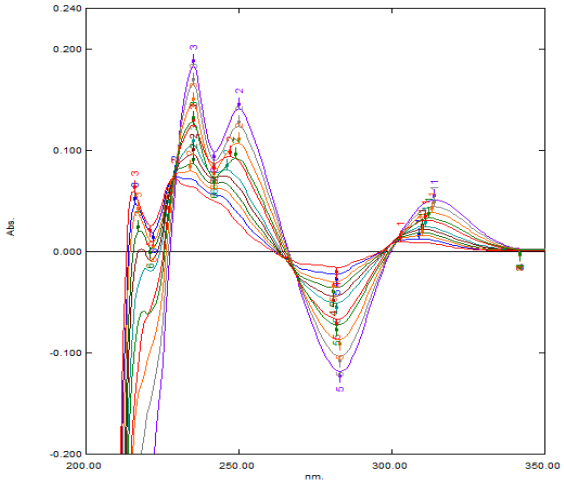

Figure (8):): The overlain spectra of the second derivative for solutions containing (4.0 – 40.0) µg/mL DicNa and fixed 15.0 µg/mL of PriM

3.2 Wavelength selection recovery for HPSAM

In the current work for (HPSAM), choosing a suitable range of the pairs of wavelengths (Table 1) known analyte quantities are sequentially new added to the mixture analyte (DicNa) and interference (PriM), and the resulting mixture absorbance was measured at the two selected wavelengths. Two straight lines were obtained and the intersection of the two linear lines is H-point (C_H, A_H), DicNa (C_H) is the analyte concentration was directly determined at the intersection of the x-axis, and (A_H) is the analytical signal by reason of the interference, resulting in high recovery percentage (99.12, and 100.12)% for (ZO-HSAM) and (2D-HPSAM) mode (Figure 9 & 10).

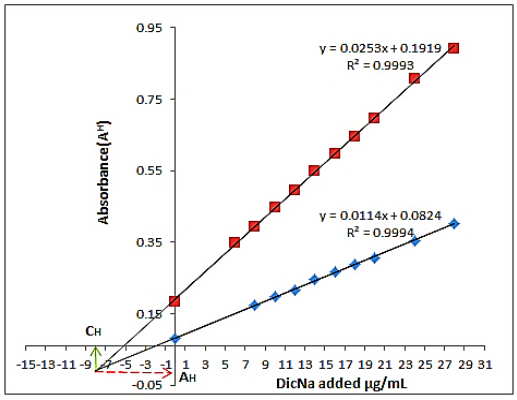

Figure (9): Plots of ZO-HPSAM method for determination of DicNa (8 µg/mL) in the presence of PriM (8 µg/mL)when different standard DicNa solutions (4-40 µg/mL) were added at the selected wavelengths 250.37 nm and 266.34 nm

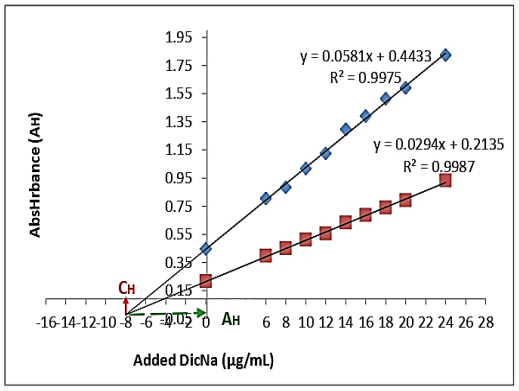

Figure (10): Figure (10): Plots of ZO-HPSAM method for determination of DicNa (8 µg/mL) in the presence of PriM (8 µg/mL) when different standard DicNa solutions (4-40 µg/mL) were added at the selected wavelengths 224.18 and 235.10 nm.

Table (1): Selection of correct wavelength for mixture analyte (DicNa) and interference (PriM) analysis

Methods	Wavelength (nm)	A-C Equation	R^2	Amount taken (µg/mL)		Amount found (µg/mL)	Recovery %	Absorbance (A_H)
				DicNa	PriM	DicNa	DicNa	PriM
ZO-HPSAM	250.37 266.34	y=0.0252x+0.1923 y=0.0114x+0.0829	0.9993 0.9994	8	8	7.93	99.12	- 0.0074
2D-HPSAM	224.18 235.10	y=0.0581x+0.4433 y=0.0294x+0.2135	0.9975 0.9987	8	8	8.01	100.12	- 0.022

3.3 Linearity studies

All methods were legalized as demonstrated by ICH guidelines (Guideline, 2005, Patil et al., 2016). The calibration curves for each one of (DicNa, and PriM) in zero-order and isosbestic point as revealed in (Figure 11 & 12) respectively, showed that the system obeyed beer's law of each component accessible linear response to the analyte concentration. For the Q-absorption ratio method way, the wavelengths designated and selected were 265nm (isosbestic point) and 285nm (λmax of DicNa) with 258nm (λmax of PriM). The absorbance at these two wavelengths for all standard solutions of both DicNa and PriM were consistent and measured, so the statistical data for these calibration curves are concise in (Table 2).

For supposed second order Derivative and zero crossing order the linearity of the methods was measured at different concentrations of DicNa, and PriM in the binary mixture in the field ranges of (4-40 and 4-30 µg/mL), respectively. Calibration graphs were constructed as shown in (Figures 13 &14). The statistical parameters are summerized in (Tables 3). All plots had an acceptable linear relationship and observed for each compound as indicated by their correlation coefficients.

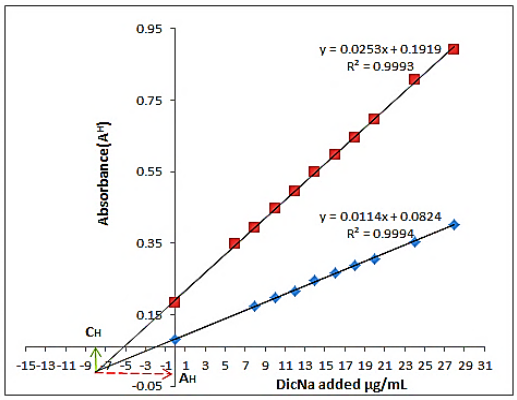

Figure (9): Plots of ZO-HPSAM method for determination of DicNa (8 µg/mL) in the presence of PriM (8 µg/mL)when different standard DicNa solutions (4-40 µg/mL) were added at the selected wavelengths 250.37 nm and 266.34 nm

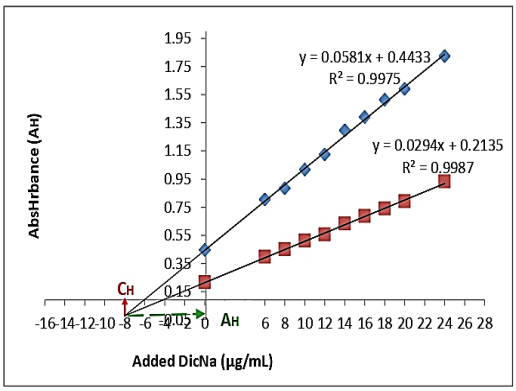

Figure (10): Figure (10): Plots of ZO-HPSAM method for determination of DicNa (8 µg/mL) in the presence of PriM (8 µg/mL) when different standard DicNa solutions (4-40 µg/mL) were added at the selected wavelengths 224.18 and 235.10 nm.

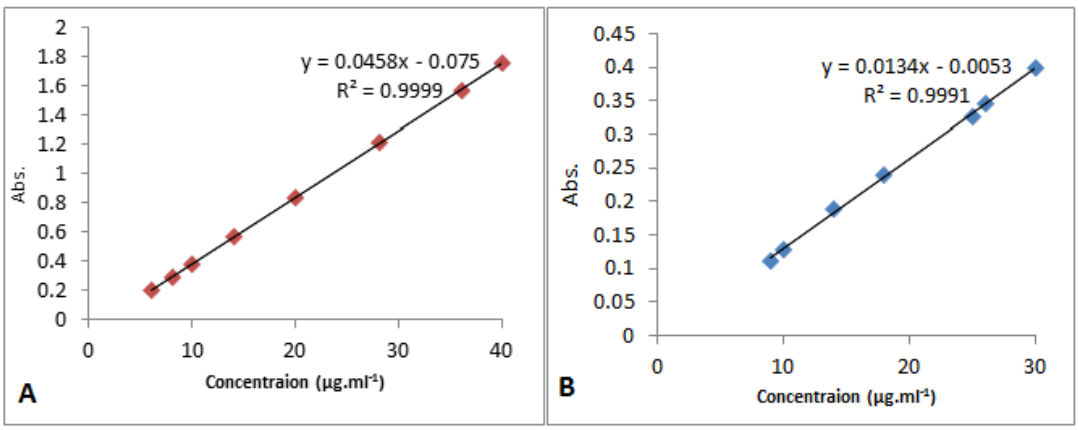

Figure (11): Linearity of zero order (A) DicNa concentration range of (4-40) µg/mL, and (B) PriM concentration range of (4-40) µg/mL

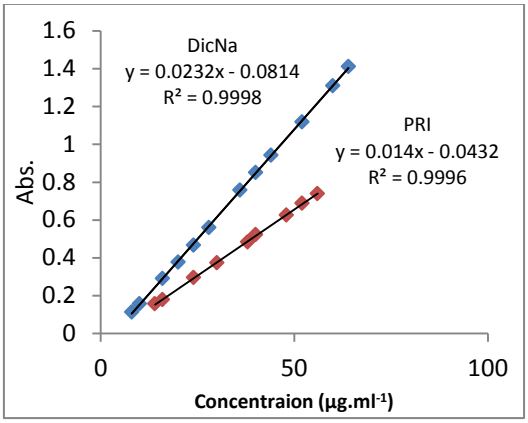

Figure (12): Linearity constructed for DicNa concentration range of (8-64) µg/mL and PriM concentration range of (14-56) µg/mL at 265nm (isosbestic point)

Table (2): Regression data of DicNa (285nm), and PriM (258nm) by Q-absorbance ratio spectrophotometric method at isosbestic point (265nm).

Parameters	Diclofenac sodium (DicNa)	Pridinol mesylate (PriM)	Q-Absorbance Ratio PriM & DicNa (isosbestic point)
λ_{max} (nm)	285	258	265
Linearity range (µg/ml)	6-40	9-30	14-56 & 8-64
Regression equation	y = 0.0458x[DicNa] - 0.075	y = 0.0134x[PriM] - 0.0053	y = 0.014x[PRI] - 0.0432 & y = 0.0232 x [DicNa] - 0.0814
Slop (L/mg.cm)	0.0458	0.0134	0.014 & 0.0232
Intercept	0.075	0.0053	0.0432 & 0.0814
Correlation coefficient (R^2)	0.9999	0.9995	0.9996 & 0.9998
LOD[a] (µg/ml)	0.6546	2.46	3.09 & 2.01
LOQ[b] (µg/ml)	1.98	7.48	9.36 & 6.10

[a] & [b]:The limit of detection (LOD) and the limit of quantification (LOQ) were derived by calculating (3.3 for LOD and 10 for LOQ) using the following equations designated by International Conference on Harmonization (ICH) guidelines. **LOD = 3.3 × σ/S…….. (3) LOQ = 10 × σ/S…… (4)** Where, σ = the standard deviation of the response and S = slope of the calibration curve.

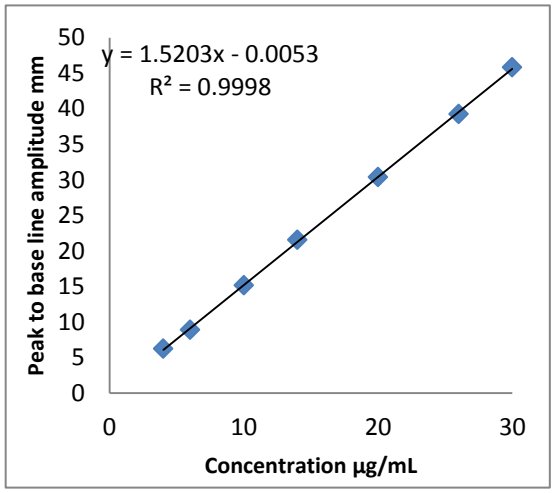

Figure (13): Calibration curve constructed under 2D proposed method for PriM in the present of fixed 20 µg/mL DicNa at 228.02nm

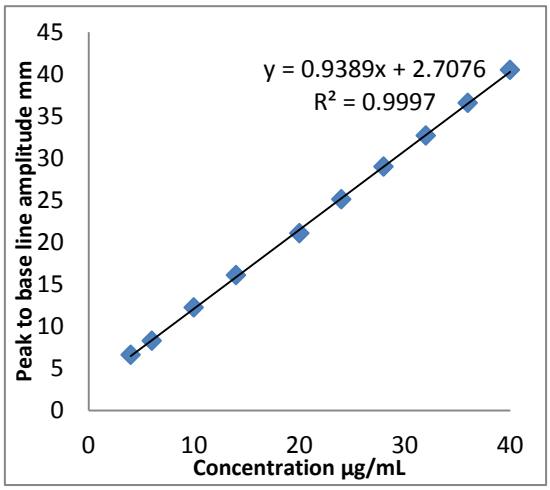

Figure (14): Calibration curves via second derivative spectra of DicNa in the present of fixed 15 µg/mL PriM at 251.69 nm.

Table (3): Statistical data of the calibration curves of the simultaneous spectrophotometric determination of DicNa, and PriM using zero-crossing technique

Compound	Methods of analysis	Linearity range (µg/mL)	Detection limit (µg/mL)	Regression equation	R^2
DicNa	Height at 251.69 nm (zero-crossing point of predinol)	4-40	1.46	Y=0.9389x+2.7076	0.9997
PriM	Height at 228.02 nm (zero-crossing point of DicNa)	4-30	0.96	y = 1.5203x - 0.0053	0.9998

3.4 Determination of unknown concentration of interference (PriM) and Analyte(DicNa) in HPSAM

In order to determine the unknown concentrations of DicNa and PriM in the mixture, it is necessary to identify the linear range which obeyed Beers law for each of them using the HPSAM method.

3.4.1 Calibration curve of the interference (PriM)

To determine the interference PriM concentration from the lineup amount of the H-point (A_H) a calibration graph is necessary. Specific DicNa concentrations were added to different mixtures of constant DicNa concentration (8 µg/mL) and different PriM concentrations (6 - 24 µg/mL). The absorbance was measured at 250.37 and 266.34 nm for (zero-order) and 224.18 and 235.10 nm for (2^{nd} order derivative) modes and plotted against added DicNa concentrations (Figure 15 & 16) separately.

A calibration curve is created by plotting the measured A_H values obtained from (Figure 14) beside the PriM concentration ranged (6.0-24.0 µg/mL) for the determination of PriM interference concentration. Calibrations are obeyed Beer's law in the UV region in the interior concentration range of (6.0-24.0) µg/mL and there was a negative deviation after 24.0 µg/mL for both (zero and second order mode) as shown in (Figure 17 & 18).

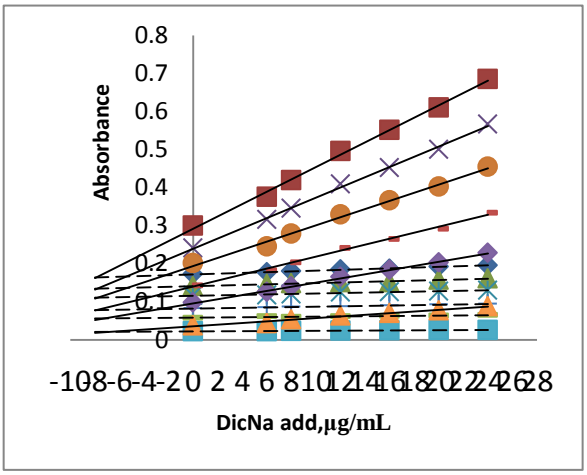

Figure (15): Plots of ZO-HPSAM for a fixed DicNa (8 µg/mL) and different concentrations of PriM at wavelengths of 250.37 and 266.34 nm

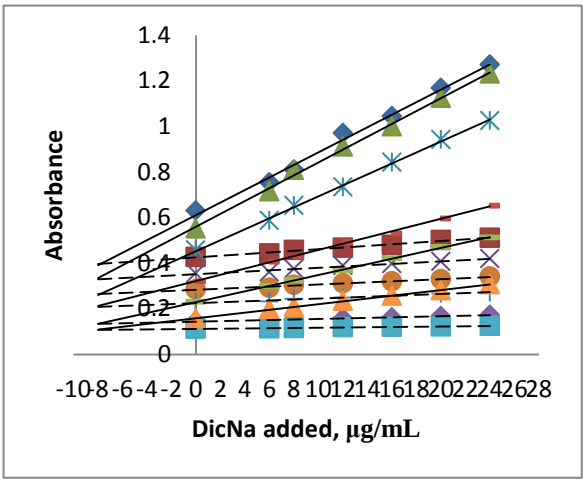

Figure (16): Plots of 2D-HPSAM for a fixed DicNa (8 µg/mL) and different PriM concentrations at wavelengths of 224.18 and λ 235.1 nm

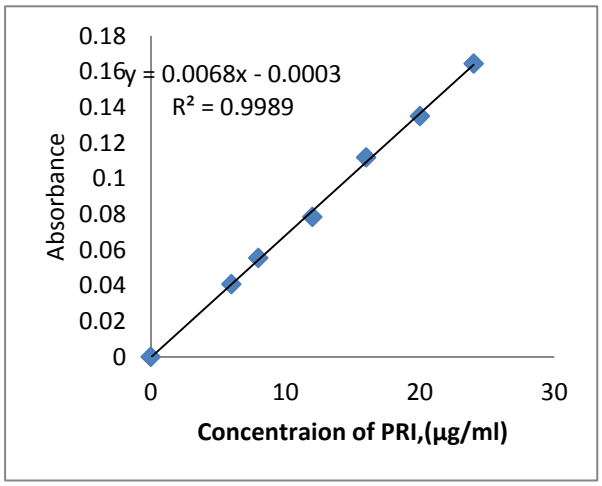

Figure (17): Calibration curve of PriM in the presence of fixed amount DicNa (8 µg/mL) for (ZO-HPSAM)

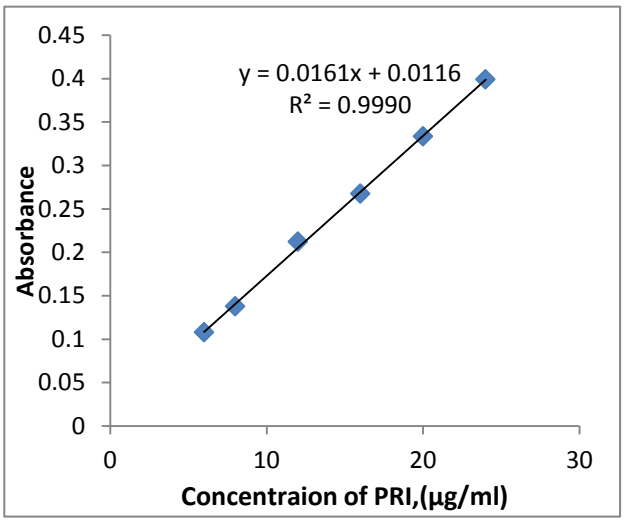

Figure (18): Calibration curve of PriM in the presence of fixed amount DicNa (8 µg/mL) for (2D-HPSAM)

3.4.2 Calibration curve of the analyte (DicNa)

To measure a quantity of the analyte DicNa, exact DicNa concentrations were added to different mixtures of constant PriM concentration (8.0 µg/mL) and variable DicNa concentrations (4.0-14.0 µg/mL). The absorbance was measured at 250.37 nm and 266.34 nm for (zero-order), and at 224.18 nm and 235.1 nm for (2^{nd} order) mode and plotted against added DicNa concentrations (Figure 19 & 20).

A calibration curve is built on plotting the founded C_H values in (Figure 16 & 22) against the DicNa concentrations ranged (4.0-14.0 µg/mL) for determination of DicNa analyte concentration. Calibration within the concentration range of (4.0-14.0) µg/mL was found to be linear and there was a positive and negative deviation after 14 µg/mL for (zero order) and (second order) modes as shown in (Figure 21 & 22) separately.

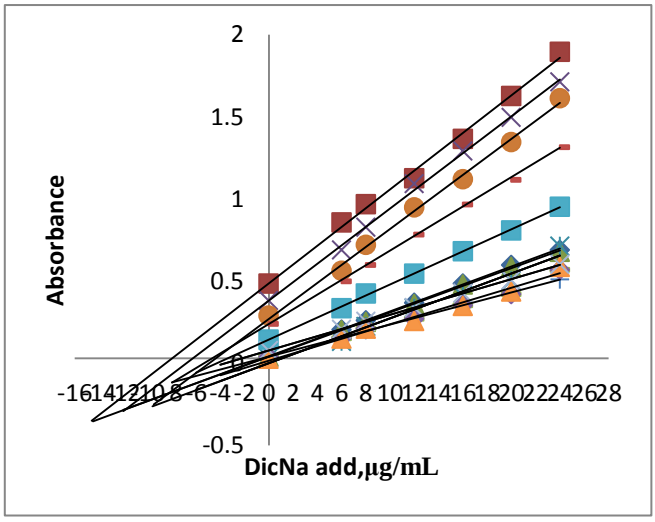

Figure (19): Plots of ZO-HPSAM curves at fixed PriM (8 µg/mL) concentration 8.0 (µg/mL) and different DicNa concentrations.

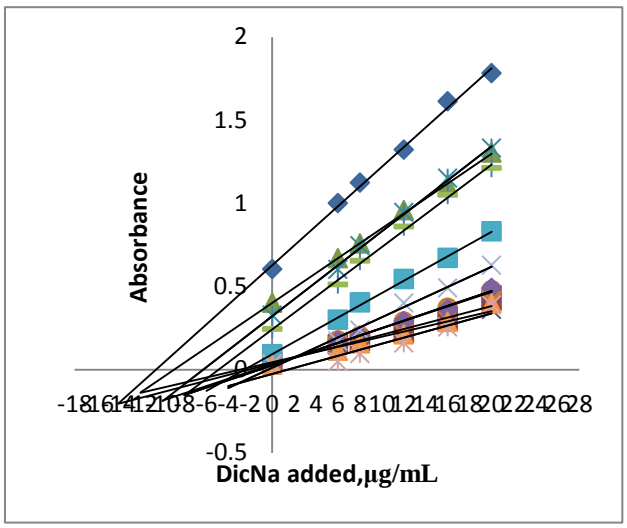

Figure (20): Plots of 2D-HPSAM curves at fixed PriM concentration (8.0 µg/mL) and different DicNa concentrations

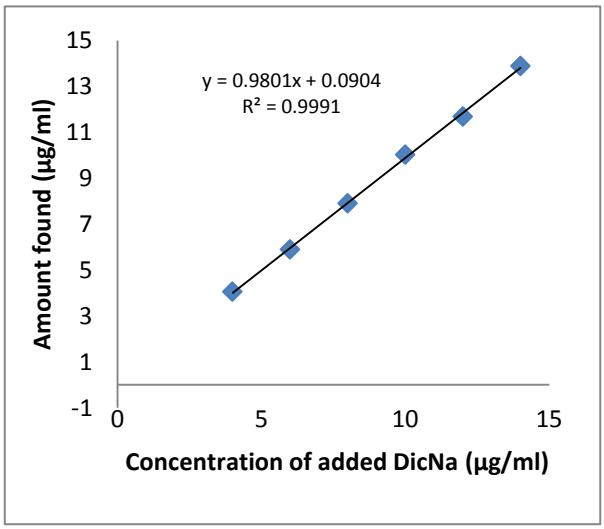

Figure (21): Calibration curve for determination of DicNa in the presence of 8 µg/mL of PriM in the mixture using ZO-HPSAM

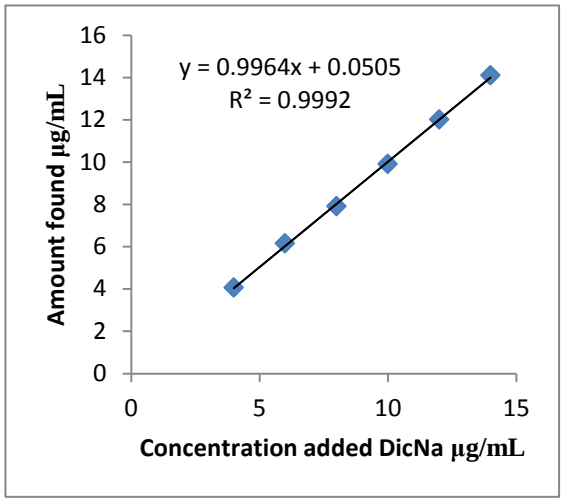

Figure (22): Calibration curve for the determination of DicNa in the presence of 8 µg/mL of PriM in the mixture using 2D-HPSAM

3.5 ACCURACY AND PRECISION

In recent years, some publications have appeared explaining several analytical titration strategies that help improve analytical accuracy (Świt et al., 2018). Both accuracy and precision of the techniques were confirmed by doing recovery studies using five replicate measurements for synthetic lab mixture and pharmaceutical formulation at three concentration levels of standard addition. The values of relative error (Error %) and relative standard deviation (RSD %) for these replicate measurements of DicNa and PriM were calculated. The results of Q-Absorption ratio and 2nd derivative spectrophotometric method zero-crossing point are tabulated in (Table 4 & 5) correspondingly.

After the determination of interference (PriM) concentration from the lineup amount of the H-point (AH) in both (ZO-HPSAM) and (2D-HPSAM,) good recovery of (97.75-102.25) percentage and (98.0-100.62) percentage (Table 6 & 7) respectively were succeeded for DicNa and PriM in the synthetic mixture, Both accuracy and precision of the method are acceptable.

(Table 8 & 9) Showed regression equations and recovery percentage for the determination of the quantity of the analyte (DicNa), a good recovery of (97.33-102.12) percent for (ZO-HPSAM) and (98.25-103.37) percent for (2D-HPSAM) were achieved for DicNa and PriM in the mixture.

Table (4): Evaluation of accuracy and precision for the determination of DicNa and PriM by Q-Absorption ratio method

Parameter	compound	λmax (nm)	Concentration (µg/mL)	Error %	RSD %
Q-Absorption ratio method	Diclofenac sodium(DicNa)	285	6	+ 3.79	+ 0.41
			20	- 4.77	+ 0.34
			40	- 1.96	+ 1.69
	Pridinol mesylate(PriM)	258	9	- 2.96	+ 4.22
			14	- 1.90	+ 4.12
			30	+ 2.91	+ 3.40
		Isosbestic point	8	- 1.40	+ 2.78
	Diclofenac sodium(DicNa)	265	32	- 4.63	+ 2.21
			64	- 4.35	+ 1.72
	Pridinol mesylate(PriM)	Isosbestic point	14	- 3.26	+ 2.65
		265	30	- 4.47	+ 2.88
			56	- 4.30	+ 4.36

Table (5): Evaluation of accuracy and precision for the determination of DicNa and PriM using 2nd derivative zero-crossings technique

Compound	Methods of analysis	Concentration (µg/mL)	Error %	RSD %
DicNa	Height at 251.69 nm (zero-crossing point PriM)	4	- 2.48	+ 2.16
		20	- 2.26	+ 3.40
		40	+ 1.75	+ 1.60
PriM	Height at 228.02 nm (zero-crossing point of DicNa)	4	+ 2.19	+ 3.46
		14	+ 3.16	+ 3.20
		30	- 0.95	+1.98

Table (6): Regression equations and recovery percentage of PriM (A_H) and a fixed concentration of DicNa (8 µg/mL) (-C_H) in (ZO-HPSAM)

A-C Equation	$\sqrt{R^2}$	Amount taken (µg/ml) DicNa	Amount taken (µg/ml) PriM	DicNa Amount found (µg/ml)	DicNa Recovery %	PriM Amount found (µg/ml)	PriM Recovery %
y=0.0021x+0.0394	0.9947	8.0	0	8.05	100.6	0	0
y=0.0001x+0.0233	0.9689						
y=0.0022x+0.0579	0.9867	8.0	6.0	7.81	97.75	6.03	100.5
y=0.0002+0.04051	0.9806						
y=0.0054x+0.0979	0.9973	8.0	8.0	7.92	99.01	8.12	101.5
y=0.0003x+0.0579	0.9731						
y=0.0078x+0.1425	0.9909	8.0	12.0	8.18	102.25	11.78	98.16
y=0.0005x+0.0826	0.9668						
y=0.0106x+0.1961	0.9952	8.0	16.0	7.95	99.37	16.27	101.68
y=0.0006x+0.1166	0.8953						
y=0.0133x+0.2412	0.9943	8.0	20.0	7.89	98.62	19.88	99.4
y=0.0008x+0.1413	0.9049						
y=0.0161x+0.2943	0.9968	8.0	24.0	8.06	100.75	24.22	100.9
y=0.001x+0.17250	0.9766						

Table (7): Regression equations and recovery percentage of PriM (A_H) and a fixed concentration of DicNa (8 µg/mL) (-C_H) in (2D-HPSAM)

A-C Equation	$\sqrt{R^2}$	Amount taken (µg/ml)		DicNa		PriM	
		DicNa	PRI	Amount found (µg/ml)	Recovery %	Amount found (µg/ml)	Recovery %
y=0.005x+0.13140	0.9985	8.0	0	7.93	99.12	0	0
y=0.0007x+0.0973	0.9975						
y=0.0062x +0.1573	0.9988	8.0	6.0	7.98	99.75	5.99	99.80
y=0.0005x+0.1118	0.9750						
y=0.0118x+0.2305	0.9980	8.0	8.0	7.86	98.25	7.84	98.0
y=0.0011x+0.1464	0.9900						
y=0.0137x+0.3208	0.9936	8.0	12.0	7.94	99.25	12.31	102.5
y=0.0019x+0.2272	0.9825						
y=0.0238x+0.4582	0.9980	8.0	16.0	8.02	100.25	15.89	99.31
y=0.0022x+0.2849	0.9929						
y=0.0292x+0.5688	0.9982	8.0	20.0	8.05	100.62	20.0	100
y=0.0027x+0.3553	0.9948						
y=0.0258x+0.6063	0.9936	8.0	24.0	8.03	100.37	24.07	100.29
y=0.0035x+0.9825	0.9825						

Table (8): Regression equations and recovery percentage of DicNa (-C_H) and a fixed concentration of PriM (8 µg/mL) (A_H) in (ZO-HPSAM)

A-C Equation	$\sqrt{R^2}$	Amount taken (µg/ml)		DicNa		PriM	
		DicNa	PriM	Amount found (µg/ml)	Recovery %	Amount found (µg/ml)	Recovery %
y=0.0415x+0.1152	0.9997	4	8	4.06	101.15	7.89	98.5
y=0.0159x+0.0112	0.9992						
y=0.0336x+0.1434	0.9994	6	8	5.9	98.3	7.95	99.37
y=0.0146x+0.0313	0.9998						
y=0.0308x+0.1875	0.9949	8	8	7.91	98.87	8.10	101.25
y=0.0144x+0.0585	0.9995						
y=0.0294x+0.2399	0.9978	10	8	10.03	100.3	8.12	101.50
y=0.0151x+0.0965	0.9862						
y=0.0287x+0.2764	0.9982	12	8	11.68	97.33	8.13	102.12
y=0.0144x+0.1106	0.9994						
y=0.0292x+0.3491	0.9925	14	8	13.89	99.21	8.08	101
y=0.014x+0.1389	0.9938						

Table (9): Regression equations and recovery percentage of DicNa (-CH) and a fixed concentration of PriM (8 µg/mL) (AH) in (2D-HPSAM)

A-C Equation	$\sqrt{R^2}$	Amount taken (µg/ml)		DicNa		PriM	
		DicNa	PriM	Amount found (µg/ml)	Recovery %	Amount found (µg/ml)	Recovery %
y=0.0323x-0.0163	0.9929	4	8	4.06	101.5	7.89	98.65
y=0.0193x-0.0676	0.9947						
y=0.0379x+0.0916	0.9964	6	8	6.15	102.5	8.06	100.75
y=0.0186x-0.0271	0.9825						
y=0.0498x+0.2422	0.9929	8	8	7.86	98.25	8.18	102.25
y=0.0221x+0.0272	0.9864						
y=0.0515x+0.3262	0.9956	10	8	9.9	99.0	8.27	10.3.37

y=0.0217x+0.0323	0.9904						
y=0.0449x+0.4016	0.9982	12	8	12.0	100.30	7.93	99.12
y=0.0153x+0.0450	0.9884						
y=0.0569x+0.6610	0.9941	14	8	14.10	100.71	8.07	100.87
y=0.0298x+0.0278	0.9881						

3.6 INTERFERENCES

The tolerance limit was describe as the concentration of the added species interference (such as lactose monohydrate, , magnesium stearate, starch, cellulose-microcrystalline, and mannitol) causing an error of more than ±5% on the analytical signal, then Before action with the analysis of the compound under study in pharmaceutical dosage forms, it was conducted to discover its effect. Samples were prepared by mixing known quantities of the investigated drugs with different quantities of mutual excipients. A good percentage recovered from the tested drugs obtained from those laboratory synthetic mixtures shows that there is no interference from these supplement additives with the methods applied. Furthermore, the accuracy of the proposed procedure has been more tested by applying the standard addition technique. The results obtained (Table 10) reveal a great degree of accuracy.

Table (10): Tolerance limit for foreign species on the determination of DicNa and PriM with the study methods

Methods	Lactose Monohydrate (850 µg/m)		Starch (850 µg/m)		Magnesium stearate (120 µg/m)		Cellulose-microcrystalline (180 µg/m)		Manitol (850 µg/m)	
	Error %	Recovery %	Error %	Recovery %	Error %	Recovery %	Error %	Recovery %	Error %	Recovery %
Q-Analysis	-3.35	103.35	+ 4.58	95.42	+ 4.52	95.48	- 4.01	104.01	95.68	+ 4.32
2D zero crossing point	+4.60	104.60	- 4.13	95.86	- 4.81	104.81	+ 4.65	104.65	103.90	- 3.90
HPSAM	+ 4.70	95.29	- 4.50	104.51	- 4.11	104.11	- 4.81	95.19	104.55	- 4.55

3.7 APPLICATION OF THE METHODS

To evaluate the analytical applicability of the planned methods, these methods and process have been applied on the pharmaceutical formulation (tab) and synthetic lab mixture, the absorbances of the sample solution i.e. A1 and A2 were noted at 258 nm (λ-max of PriM) and 265nm (Isosbestic point) respectively, and ratios of absorbance were calculated, i.e. A2/A1. The relative concentration of both two drugs in the sample was intended using equations [1] and [2]. The Q-analysis technique procedure was effectively used to determine the amounts of PriM and DicNa by repeated three times within the synthetic lab mixture and pharmaceutical formulation (Table 11), The results gotten were in good agreement with the relevant quantitative markers and labeled amount.

The suggested 2D zero-cross technique for simultaneous determination of DicNa, and PriM was effectively applied with the support and aid of standard addition method for simultaneous quantification of DicNa, and PriM in the pharmaceutical formulation. The results of the recovery study and application are potted in (Table 12 & 13) respectively. The recoveries ranged from 99.37% to 101.54 % at different drug concentrations, with % RSD < 2.

For the (ZO-HPSAM) and (2D-HPSAM) methods for the simultaneous estimation of DicNa and PriM in the synthetic mixture and pharmaceutical formulation. The results data are listed in (Table 14 & 15). The good agreement between the findings and the composition values suggested by the suppliers indicates that HPSAM is effectively applicable to the simultaneous evaluation of DicNa and PriM.

Table (11): Recovery study data of PriM and DicNa by (Q-Abbsorbance) method

Formulation	PriM	DicNa	PriM	DicNa

	(µg/ml)	(µg/ml)	Mean recovery [a] ± % RSD	Mean recovery [a] ± % RSD
Synthetic mixture	16	16	98.30 ± 1.05	101.21 ± 3.40
	28	28	99.69 ± 1.55	100.16 ± 2.70
	56	56	99.46 ± 1.05	99.75 ± 1.84
Tablet formulation [b]	24	24	100.64 ± 0.36	100.01 ± 0.63

[a] Average of 3 determination. [b] (Dioxaflex Plus)

Table (12): Determination of DicNa in pharmaceutical formulation by 2nd derivative zero-crossing method

Drug sample taken		Standard addition technique		
Name	µg/mL	Pure added	µg/mL	Mean recovery [a] ± % RSD
Tablet formulation [b]	8	DicNa	6	100.92 ± 1.2663
			12	100.32± 1.8112
			30	99.37 ± 0.4480
	10	PriM	7	99.78 ± 1.9171
			11	101.54± 1.0402
			18	100.09 ± 0.7143

[a] Average of 3 determination. [b] (Dioxaflex Plus)

Table (13): Simultaneous determination and recovery of DicNa, and PriM in tablet sample

Tablet sample	Ac	Method of analysis	Amount taken (µg/mL)	Amount found (µg/mL)	Recovery %
Tablet formulation	DicNa	2D zero-cross technique at 251.69 nm	50	50.39	100.78
	PriM	2D zero-cross technique at 228.02 nm	4	3.95	98.75

Table (14): Application of ZO-HPSAM for determination of DicNa and PriM in a synthetic lab mixture and pharmaceutical product

Formulation	A-C Equation	R^2	Amount taken (µg/ml)		DicNa		PriM	
			DicNa	PriM	Amount found (µg/ml)	Recovery %	Amount found (µg/ml)	Recovery %
Synthetic mixture	y=0.0291x+0.0807 y=0.0167x+0.0252	0.9953 0.9985	4.5	7.5	4.47	99.35	7.25	96.65
	y=0.0618x+0.3692 y=0.0206x+0.057	0.9884 0.9807	7.5	15	7.57	100.93	14.60	97.33
	y=0.0679x+0.584 y=0.0251x+0.1108	0.9895 0.9735	11	24	11.05	100.54	24.45	101.85
Tablet formulation	y=0.0095x+0.1748 y=0.0011x+0.0925	0.9969 0.9886	10	12	9.8	98.00	12.05	100.40

Table (15): Application of 2D-HPSAM for determination of DicNa and PriM in a synthetic lab mixture and pharmaceutical product

Formulation	A-C Equation	R^2	Amount taken (µg/ml)		DicNa		PriM	
			DicNa	PriM	Amount found (µg/ml)	Recovery %	Amount found (µg/ml)	Recovery %
Synthetic mixture	y=0.0317x+0.0042	0.9939	6	11	6.08	101.33	10.99	99.90
	y=0.0197x-0.0688	0.9959						
	y=0.0352x+0.1255	0.9977	12	18	12.05	100.41	17.78	98.77
	y=0.0221x-0.0323	0.9891						
	y=0.0574x+0.1963	0.9981	10	22	9.88	98.8	22.32	101.45
	y=0.0319x-0.0558	0.9944						
Tablet formulation	y=0.0293x+0.5664	0.9981	8	20	7.94	99.25	20.02	100.12
	y=0.0027x+0.3553	0.9901						

4. STATISTICAL ANALYSIS

Statistical study was achieved on the results found by the proposed ways and the stated reported method (Vignaduzzo et al., 2010), for each compound using Student's t- and F- tests at P = 0.05, with respect to both precision and accuracy; No significant difference was initiated, as shown in (Table 16). One-way ANOVA was applied to compare the alterations between the developed methods, (Table 17) showing no significant difference between the DicNa and PriM identification methods. Data analysis was performed by SPSS.

Table (16): Statistical comparison of the proposed methods with the reported one (Vignaduzzo et al., 2010) for determination of DicNa and PriM in their pharmaceutical formulation

Methods	Q-Absorption ratio method		2D- zero crossing		ZO-HPSAM		2D-HPSAM		* Reported method	
	DicNa	PriM	DicNa	PriM	DicNa	PriM	DicNa	PriM	DicNa	PriM
Mean	100.28	99.52	100.35	100.04	99.71	99.06	99.94	100.10	95.72	96.90
%RSD	0.6393	0.9664	0.6978	1.1520	1.3244	2.4988	1.1449	1.0986	0.9470	1.1976
n	4	4	4	4	4	4	4	4	4	4
Student t-test ** (2.306)	0.779	0.198	0.8037	0.5883	0.3431	0.2527	0.4664	0.5404		
F-value ** (6.38)	0.5003	0.6869	0.5969	0.9861	2.1225	4.5493	1.5940	0.8973		

* HPLC reported method (Vignaduzzo et al., 2010), the determinations were carried out on a Luna C18 analytical column (250mm ×4.6mm I.D., 5 mm particle size) thermostatted at 30°C. The mobile phase was a 48:9:43 (v/v/v) mixture of MeOH, 2-propanol and 50mM sodium phosphate (pH =5.5), pumped at 1.0 $mL.min^{-1}$. Detection wavelength was 225 nm. All samples were filtered through 0.45 mm nylon filters before injection.

** Theoretical values of t and F at (P= 0.05).

Table (17): Results of ANOVA (one-way) for comparison of the proposed methods and the reported (Vignaduzzo et al., 2010) for determination of DicNa and PriM in their pharmaceutical formulation

Variation	SS	df	MS	F	P-Value	F crit
DicNa						
Between Groups	4.6167	3	1.538906	0.287595	0.833494	3.490295
Within Groups	64.2113	12	5.350947			
Total	68.8280	15				

PriM						
Between Groups	4.5091	3	1.5030	0.347193	0.791871	3.490295
Within Groups	51.9498	12	4.3291			
Total	56.459	15				

5. CONCLUSION

Four exceptional exact mathematical treatments of spectral recorded data were successfully applied to determine two components DicNa and PriM all together in the laboratory prepared mixtures and the pharmaceutical formulations. Q-absorbance ratio, 2D- zero-crossing technique, HPSAM, were used to resolve and decide the overlapped spectra without previous separation of the mixtures, interferences were removed by choosing the most valid wavelength. Compared to the HPLC technique, these spectrophotometric methods did not need an early separation steps, and using toxic organic solvent, thus the method can be considered as an environmental friendly.

Authentication of the methods was established accuracy, precision, linearity, LOD, LOQ and selectively, the methods were confirmed to be selective, accurate, precise, cooperative, and effective for the analysis study of these compound drugs in a pharmaceutical dosage forms. The results were developed by four expected proposed methods, showing acceptable results compared with the reported methods. Based on the results gained and statistical data analysis, these methods are appropriate for the estimation and determination of these drugs without any interference of the pharmaceutical excipients present in the formulation and can be simply applied in acceptance sampling quality control lab.

ACKNOWLEDGEMENT

I would like to express my deep and sincere thankfulness to the Pharmaceutical Chemistry Department, College of Pharmacy, Hawler Medical University, and Ministry of Science & Technology (Iraq-Baghdad) for use of their research lab for UV-spectrophotometer and HPLC.

6. REFERENCES

ABDEL SHAHEED, C., MAHER, C., WILLIAMS, K. & MCLACHLAN, A. 2017. Efficacy and tolerability of muscle relaxants for low back pain: systematic review and meta-analysis. *European Journal of Pain*, 21**,** 228-237.

AFKHAMI, A., BAHIRAEI, A. & MADRAKIAN, T. 2016. Gold nanoparticle/multi-walled carbon nanotube modified glassy carbon electrode as a sensitive voltammetric sensor for the determination of diclofenac sodium. *Materials Science and Engineering: C*, 59**,** 168-176.

ANDRES, J. V., REIG, F. B. & FALCó, P. C. J. A. 1995. H-point standard additions method for analyte determination in ternary mixtures. 120**,** 299-304.

ANGGRAINI, Y. & EKAWATI, I. W. 2020. Acupressure therapy as a pain reliever for dysmenorrhea. *Enfermería Clínica*, 30**,** 84-87.

BREZOVSKA, M., JAMPILEK, J. & OPATRILOVA, R. J. C. P. A. 2013. A review of HPLC methods used for determining the presence of meloxicam. 9**,** 69-76.

CHETHANA, B., BASAVANNA, S., ARTHOBA NAIK, Y. J. I. & RESEARCH, E. C. 2012. Voltammetric determination of diclofenac sodium using tyrosine-modified carbon paste electrode. 51**,** 10287-10295.

DARWEESH, S. A., KHALAF, H. S., AL-KHALISY, R. S., YASEEN, H. M. & MAHMOOD, R. M. 2018. Advancement and validation of new Derivative spectrophotometric method for individual and simultaneous estimation of Diclofenac sodium and nicotinamide. *Oriental Journal of Chemistry*, 34**,** 1625.

DIKRAN, S. B. & MAHMOOD, R. M. 2017. Spectrophotometric Determination of Diclofenac sodium Using 2, 4-dinitrophenylhydrazine in Pure Form and Pharmaceutical Preparations. *Ibn AL-Haitham Journal For Pure and Applied Science*, 28**,** 129-141.

GUIDELINE, I. H. T. J. Q. 2005. Validation of analytical procedures: text and methodology. 1**,** 05.

GUNJI, R., NADENDLA, R. R., PONNURU, V. S. J. I. J. O. D. D. & RESEARCH 2012. Simultaneous UV-spectrophotometric determination and validation of diclofenac sodium and rabeprazole sodium using hydrotropic agents in its tablet dosage form. 4**,** 316-324.

KHAMAR, J. C. & PATEL, S. A. 2012. Q-absorbance ratio spectrophotometric method for the simultaneous estimation of rifampicin and piperine in their combined capsule dosage.

KIMUAM, K., RODTHONGKUM, N., NGAMROJANAVANICH, N., CHAILAPAKUL, O. & RUECHA, N. 2020. Single step preparation of platinum nanoflowers/reduced graphene oxide electrode as a novel platform for diclofenac sensor. *Microchemical Journal*, 155**,** 104744.

KLEEMANN, A., ENGEL, J., KUTSCHER, B., REICHERT, D., KLEEMANN, A., ENGEL, J., KUTSCHER, B. & REICHERT, D. 2014. *Pharmaceutical substances, 2009: Syntheses,*

Patents and Applications of the most relevant APIs, Georg Thieme Verlag.

KUS, S., MARCZENKO, Z. & OBARSKI, N. J. C. A. 1996. Derivative UV-VIS spectrophotometry in analytical chemistry. 41**,** 889-927.

LINS, P. V. S., HENRIQUE, D. C., IDE, A. H., DA SILVA DUARTE, J. L., DOTTO, G. L., YAZIDI, A., SELLAOUI, L., ERTO, A., E SILVA, C. L. D. P. & MEILI, L. 2020. Adsorption of a non-steroidal anti-inflammatory drug onto MgAl/LDH-activated carbon composite–Experimental investigation and statistical physics modeling. *Colloids and Surfaces A: Physicochemical and Engineering Aspects,* 586**,** 124217.

MANE, R. V., PATEL, K., SUSHMITHA, G. S. & VASANTHARAJU, S. 2019. Development and Validation of Diclofenac sodium in tablets using Simple UV Spectrophotometric method. *Research Journal of Pharmacy and Technology,* 12**,** 611-614.

MICHOPOULOS, A., FLOROU, A. B. & PRODROMIDIS, M. I. J. E. 2015. Ultrasensitive Determination of Vitamin B12 Using Disposable Graphite Screen-Printed Electrodes and Anodic Adsorptive Voltammetry. 27**,** 1876-1882.

MOORE, N., DUONG, M., GULMEZ, S. E., BLIN, P. & DROZ, C. 2019. Pharmacoepidemiology of non-steroidal anti-inflammatory drugs. *Therapies,* 74**,** 271-277.

O'HAVER, T. & GREEN, G. J. A. C. 1976. Numerical error analysis of derivative spectrometry for the quantitative analysis of mixtures. 48**,** 312-318.

PATEL, K. N., PATEL, J. K., RAJPUT, G. C. & RAJGOR, N. B. J. D. P. L. 2010. Derivative spectrometry method for chemical analysis: A review. 2**,** 139-150.

PATIL, P. A., RAJ, H. A. & SONARA, G. B. 2016. Q-absorbance ratio spectrophotometric method for simultaneous determination of atenolol and ivabradine hydrochloride in synthetic mixture. *Pharmaceutical and Biological Evaluations,* 3**,** 224-230.

REIG, B. J. A. 1990. F.; Campins Falco, P. 115**,** 111.

ROBINSON, J. W., FRAME, E. S. & FRAME II, G. M. 2014. *Undergraduate instrumental analysis*, CRC press.

SALAZAR-ROJAS, D., INTILANGELO, A., VIGNADUZZO, S. E., MAGGIO, R. M. J. J. O. P. & ANALYSIS, B. 2019. Development and validation of a green method for dissolution monitoring of pharmaceutical combinations. Meloxican and pridinol. 170**,** 228-233.

ŚWIT, P., VERDú-ANDRES, J., WIECZOREK, M., KOZAK, J., KOŚCIELNIAK, P. & CAMPíNS-FALCó, P. 2018. New calibration model: combining integrated calibration method and H-point standard addition method to detect and avoid interference effects. *Analytical Letters,* 51**,** 1194-1207.

TIWARI, D., LALHRIATPUIA, C. & LEE, S.-M. 2015. Hybrid materials in the removal of diclofenac sodium from aqueous solutions: Batch and column studies. *Journal of Industrial and Engineering Chemistry,* 30**,** 167-173.

VIGNADUZZO, S. E., CASTELLANO, P. M. & KAUFMAN, T. S. 2010. Experimentally designed, validated hplc simultaneous determination of pridinol and diclofenac in their combined pharmaceutical formulations, which allows limiting diclofenac related compound A. *Journal of liquid chromatography & related technologies,* 33**,** 1720-1732.

VIGNADUZZO, S. E., VERA-CANDIOTI, L., CASTELLANO, P. M., GOICOECHEA, H. C. & KAUFMAN, T. S. J. C. 2011. Multivariate optimization and validation of a CZE method for the analysis of pridinol mesylate and meloxicam in tablets. 74**,** 609-617.

WONGRAKPANICH, S., WONGRAKPANICH, A., MELHADO, K., RANGASWAMI, J. J. A. & DISEASE 2018. A comprehensive review of non-steroidal anti-inflammatory drug use in the elderly. 9**,** 143.

YANG, Y. J., LIU, X. W., KONG, X. J., QIN, Z., LI, S. H., JIAO, Z. H. & LI, J. Y. 2019. An LC–MS/MS method for the quantification of diclofenac sodium in dairy cow plasma and its application in pharmacokinetics studies. *Biomedical Chromatography,* 33**,** e4520.