



## Spectrophotometric micro-determination of three quinolones antibacterial drugs in pure and in pharmaceutical dosage forms by reactions with diphenylamine sulphonate redox indicator

\*Zayed M. A and Belal R. M

Chemistry Department, Faculty of Science, Cairo University, 12613- Giza, Egypt

Received: 04-11-2014 / Revised: 21-11-2014 / Accepted: 23-11-2014

### ABSTRACT

In the present study, reliable, sensitive and efficient spectrophotometric methods for the determination of three quinolones, namely Ciprofloxacin (CP), Norfloxacin (NOR) and Nalidixic acid (NA) have been performed either in pure or in pharmaceutical dosage forms. The methods are based on the reaction of the studied drugs with diphenylamine sulphonate (DPAS) indicator in its oxidized form obtained by titration with potassium dichromate in sulfuric acid medium. For a first time, DPAS is used as an oxidant spectrophotometric self-indicator. It involved follow DPAS reactions with quinolones; where two reaction products are formed in two concentration ranges of each drug; and two mechanisms of reactions are involved. In the first reaction mechanism drugs reduce DPAS oxidant (violet form) and the concentrations are micro-determined by absorbance measurement at concentration ranges 11.6 – 92.9, 19.3– 154.3 and 15.9– 127.7  $\mu\text{gml}^{-1}$  and  $\lambda_{\text{max}} = 545 - 550 \text{ nm}$  for NA, CIP and NOR respectively. In the second mechanism their concentrations are micro-determined via ion pair formation (brown form) after 30 min and measured at concentration ranges 2.3 – 9.3, 1.9 – 15.4 and 1.6– 12.7  $\mu\text{gml}^{-1}$  and  $\lambda_{\text{max}} = 245, 280, \text{ and } 285 \text{ nm}$  for NA, CIP and NOR respectively. The results of two reaction mechanisms are compared and validated statistically by % recovery and SD values. The values of % recovery are found to be 99.98, 99.86, and 100.58 and those of SD are 0.33, 0.24, and 0.31 in violet forms; while % recovery = 99.65, 99.7 and 99.95; SD = 0.011, 0.012 and 0.032 in brown forms for CIP, NA and NOR respectively. The robustness and ruggedness of the results obtained by the second mechanism are checked by inter and intra-days results. The whole results obtained by the two mechanisms are also found to be in good agreement with those given by the official methods as confirmed by F- and t- tests.

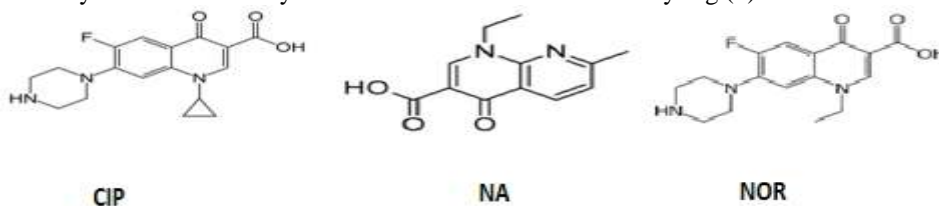
**Key Words:** Quinolones, Ciprofloxacin, Norfloxacin, Nalidixic acid, Diphenyl amine sulfonate (DPAS), Spectrophotometric methods



### INTRODUCTION

Nalidixic acid (NA), Ciprofloxacin (CIP), and Norfloxacin (NOR) are antimicrobial agents belonging to 4-Quinolone. 4-Quinolone antibiotics are characterized by their ability to inhibit the replication of DNA gyrase (Topoisomerase) which is essential for the reproduction of bacterial DNA<sup>[1]</sup>. They are commercially available for treatment

of a wide range of infections. The fluorinated 4-quinolone derivatives have a broad spectrum activity and are more potent in-vitro than the non-fluorinated ones. The widespread use of this group of drugs has prompted extensive literature on their analysis in dosage forms and biological fluids. The structural formulas of the selected drugs are given by Fig (1).



**Fig (1):** The structural formulas of Ciprofloxacin (CIP), Nalidixic acid (ND) and Norfloxacin (NOR)

The United States Pharmacopoeia<sup>[2]</sup> recommends a non-aqueous titration method for the determination of nalidixic acid, a liquid chromatographic method for determination of ciprofloxacin and a non-aqueous titrimetric method, with potentiometric detection of the end point for Norfloxacin, in their bulk; and spectrophotometric or HPLC methods for their dosage forms. Also, the British Pharmacopoeia<sup>[3]</sup> reported similar titrimetric methods for nalidixic acid, norfloxacin and ciprofloxacin in pure forms; and a spectrophotometric method for their dosage forms. Spectrophotometric methods reported for the determination of the studied drugs included oxidative coupling with 3-methyl-2-benzothiazolinone hydrazone hydrochloride (MBTH) and cerium (IV) ammonium sulphate<sup>[4]</sup>. Ion-pair complex formation with xanthenes dyes<sup>[5]</sup>, cobalt (II) tetrathiocyanate<sup>[6]</sup>, nickel (II) tetra thiocyanate<sup>[7]</sup>, and bromocresol green<sup>[8]</sup> had been reported. Also charge-transfer complexation with the acid-dye bromocresol green<sup>[9]</sup> and  $\pi$ -acceptors such as tetracyanoethylene and chloranilic acid<sup>[10]</sup> had been studied. Complexation with iron (III), copper (II) ions<sup>[11, 12]</sup> or with tris (o-phenanthroline) iron (II) and tris (bipyridyl) iron (II)<sup>[13]</sup>. Spectrophotometric study presented the kinetics and degradation pathways of oxidation of the studied drugs by permanganate in alkaline medium<sup>[14,15]</sup>. Derivative spectrophotometric analysis<sup>[16-18]</sup> had been reported. Other methods included potentiometry<sup>[19-22]</sup> and the oxidation reaction of norfloxacin with cerium (IV)<sup>[23]</sup>, voltammetry<sup>[24]</sup>, titrimetry<sup>[25-27]</sup>, atomic absorption spectroscopy (AAS) via reaction with metal ions<sup>[28]</sup>, fluorimetry<sup>[29]</sup>, UPLC<sup>[30]</sup>, HPLC<sup>[31-33]</sup>, GC<sup>[34]</sup> had been reported for the determination of the studied drugs. This paper aims chiefly to find simple spectrophotometric methods for the micro-determination of the above mentioned drugs in pure forms and their tablet formulations based on the redox reaction by using DPAS oxidant indicator, and following the reactions through Visible region to their completion at UV region including the studying of the optimum conditions for the reactions to take place.

## EXPERIMENTAL

**Materials and Reagents:** All materials used were of analytical reagent grade and some of them were used as such without any further purification. They included Nalidixic acid provided by Applichem-Germany, Ciprofloxacin (CIP) provided by Unipharma, Egypt, and Norfloxacin (NOR) provided by Egyptian International Pharmaceutical Industries CO. (EIPICO) – Egypt. Stock solutions of the studied drugs were prepared as  $10^{-3}$ M. Where CIP was prepared by dissolving the

accurately weighed amount of the pure drug, NA was prepared by dissolving the accurately weighed amount of the pure drug in 0.05 M NaHCO<sub>3</sub> solution with gentle warming, NOR was prepared by dissolving the accurately weighed amount of the pure drug in 40 mL NaHCO<sub>3</sub> solution with heating for 10 minutes, and finally the volumes were completed to 100 mL measuring flask by distilled water. The solutions were stable for at least two weeks if they had been stored in a cool (< 25 °C) and dark place. Sodium Diphenylamine Sulphonate (DPAS) supplied from Alpha chemika – India, and was prepared in distilled water as  $10^{-3}$ M. Sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) was supplied from Merck, and was prepared in distilled water as 2N. Potassium dichromate (K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>) was supplied from Adwic, and was prepared in distilled water as 0.1M. Solutions of lower concentration were obtained by accurate dilution with distilled water. Ciprofar tablets were obtained from Pharco Pharmaceuticals, Egypt, labeled to contain (500 mg CIP/ tablet). Nalidram tablets were obtained from Memphis Co. Pharm. and Chemical Industry, Egypt, labeled to contain (500 mg NA / tablet). Norbactin tablets were obtained from CID Pharmaceuticals Co, Egypt, labeled to contain (400 mg NOR / tablet).

**Apparatus:** Optizen recording UV-Visible spectrophotometer (Model 5u470 / pop127022-00) auto sampler system, equipped with 1 cm matched quartz cells was used for spectrophotometric measurements. Weights measurement was performed by using Radwag wagi Elektroniczne Sensitive analytical balance 0.0001g, Model: AS 220/C/1. Stirring and heating were performed by using ARE Heating Magnetic Stirrer Thermostated Hot Plate, Model: VELP-Europe. Automatic Micropipettes, Model: Accupipette USA, Volume range 100-1000  $\mu$ L were used to measure the small volumes.

## General Recommended Procedure

**Procedure for drugs in pure form:** Solution of DPAS in its oxidized form (Blank) was prepared by its titration in 0.2 N H<sub>2</sub>SO<sub>4</sub> against 0.1M K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> till violet color. Solutions of equimolar amounts ( $4 \times 10^{-4}$  M) were prepared between the studied drugs and DPAS indicator in its oxidized form and spectrophotometric determinations were carried out at visible region (instantaneously) and after dilution to ( $4 \times 10^{-5}$ M) at UV- region (after 30 min.).

**Procedure for dosage forms:** Each ten tablets of Ciprofar (500 mg/tablet), Nalidram (500 mg/tablet), and Norbactin (400 mg/tablet) were weighed and powdered well. Equivalent amount of

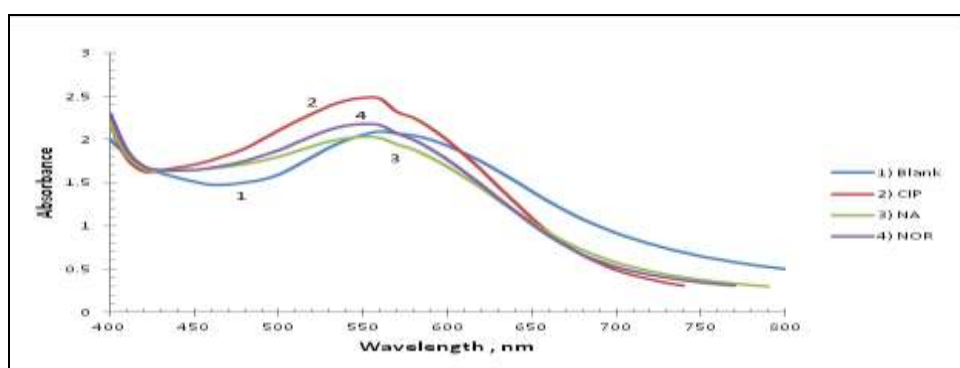
powder to one tablet of the drugs was weighed, and dissolved in sufficient amount of 0.05 M NaHCO<sub>3</sub> solution, with gentle warming. The resulting solutions were shaken well. The solutions of the drugs were transferred into 100 mL volumetric flask and the volume completed to the mark with distilled water. Analysis was completed as previously mentioned under the general procedure to be measured in both Vis and UV regions. The nominal content of the tablets was thus calculated either from a previously plotted calibration graph or using the regression equation.

## RESULT AND DISCUSSION

Usually DPAS used as an indicator to follow redox reaction between oxidant like K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> and

reducing agents like ferrous in sulfuric acid medium; aiming to detect end-point in volumetric titrations [35]. In this paper DPAS is used as an oxidant in its oxidized form and as spectrophotometric self-indicator, for a first time, to follow its reaction with fluoroquinolones such as ciprofloxacin (CIP), Norfloxacin (NOR) and Nalidixic acid (NA) spectrophotometrically.

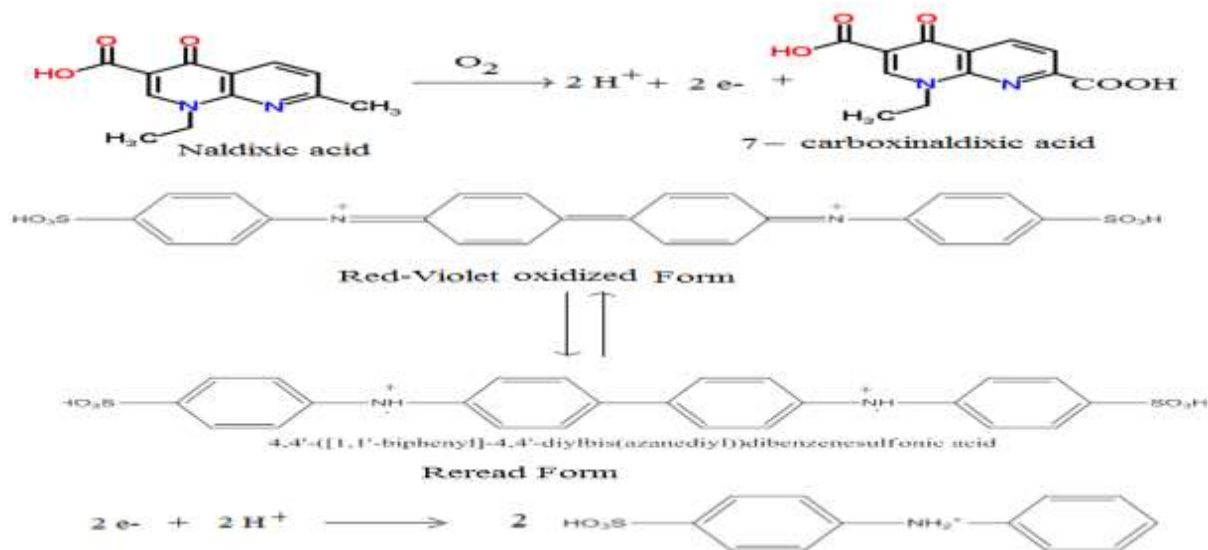
**Violet Form and selection of suitable wavelengths:** In order to prepare DPAS in its oxidized form; it is titrated against K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> in sulfuric acid medium till violet color. The obtained results are given in Fig (2); it has a λ<sub>max</sub> = 560 nm (curve1). It is shifted to lower wavelengths on reaction with the cited drugs at the same conditions within 10 minutes (curves 2, 3, 4).



**Fig (2):** Visible spectra of  $4 \times 10^{-4}$  M DPAS oxidant indicator as a blank (curve 1) and  $4 \times 10^{-4}$  M mixture of DPAS oxidant, with drug CIP, NA or NOR (curves 2-4) at normal temp.

These results indicate that, the reaction between DPAS in its oxidized form and the cited drugs in its reduced form can be followed at 550 nm for both CIP and NOR while at 545 nm for NA due to the proposed redox reaction mechanism presented in

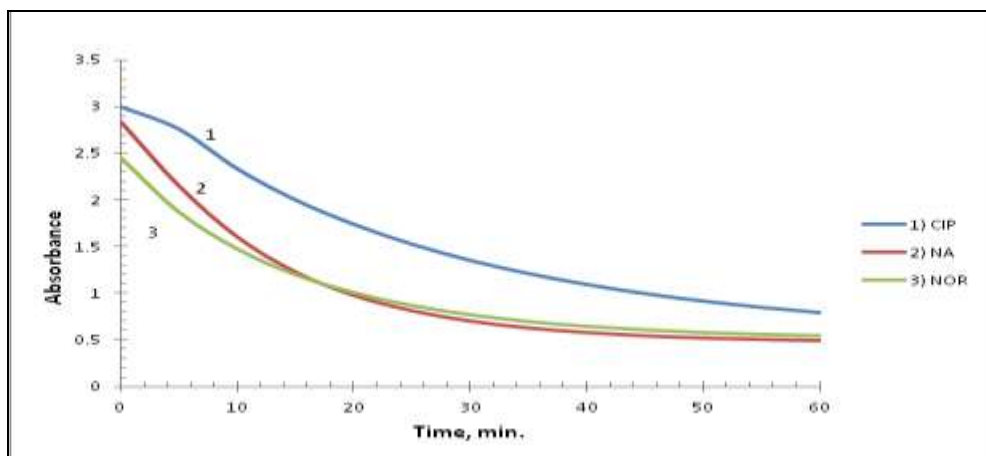
Scheme 1, taking Nalidixic acid as an example. In this scheme; the NA oxidized to 7-carboxinaldixic acid and DPAS changed to its reduced form coming from reduction of quinoid structure via reaction with the drug.



**Scheme 1.** The proposed redox reaction of DPAS oxidant and NA drug reductant in acid medium

**Effect of time:** It was noticed that the violet reaction product formed between DPAS oxidized form indicator and the cited drug had greatly affected by time where the violet colored products absorbance had been decayed with time and their

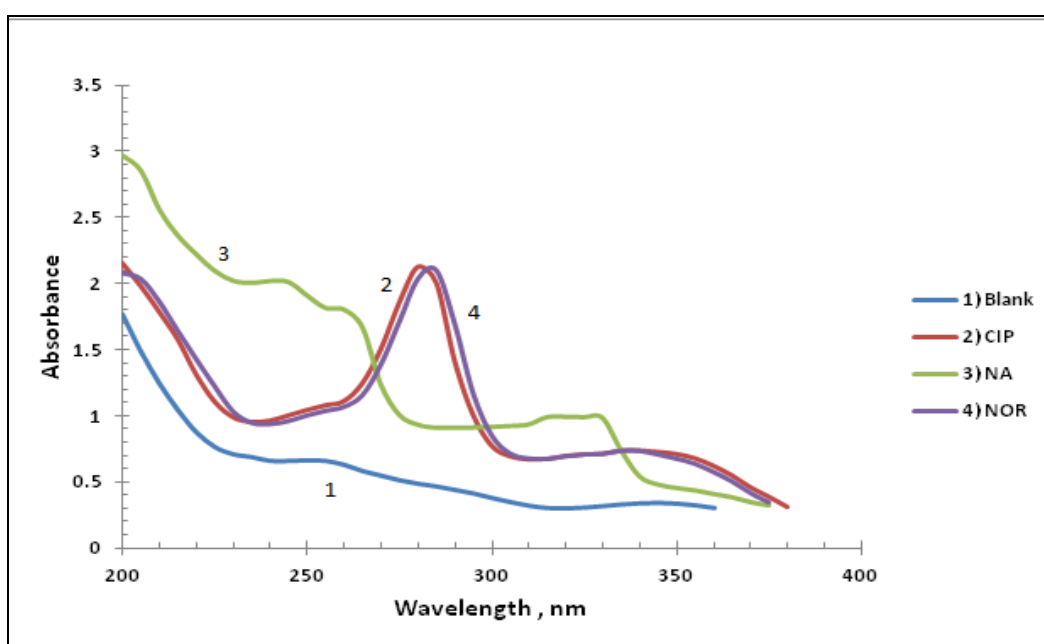
spectra had been shifted into UV region as illustrated in Fig (3). This decay may be attributed to the change of DPAS from violet oxidized form into a brown reread form of  $\lambda_{max}$ , studied in UV region.



**Fig (3):** Effect of time at normal temp on the spectra of  $4 \times 10^{-4} \text{M}$  mixture of DPAS oxidant, with drug: 1) CIP (550nm), 2) NA (545nm), or 3) NOR (550nm)

**Brown Form and selection of suitable wavelengths:** Spectral studies were carried out on the change of DPAS indicator into a brown reread form (curve 1) and its reaction with CIP (curve 2), NA (curve 3), and NOR (curve 4), as shown in Fig (4). It is obvious from the data in Fig 4 that, the  $\lambda_{max}$  of DPAS indicator is shifted from 560 nm (violet form) Figure (1) to 250 nm (brown form).

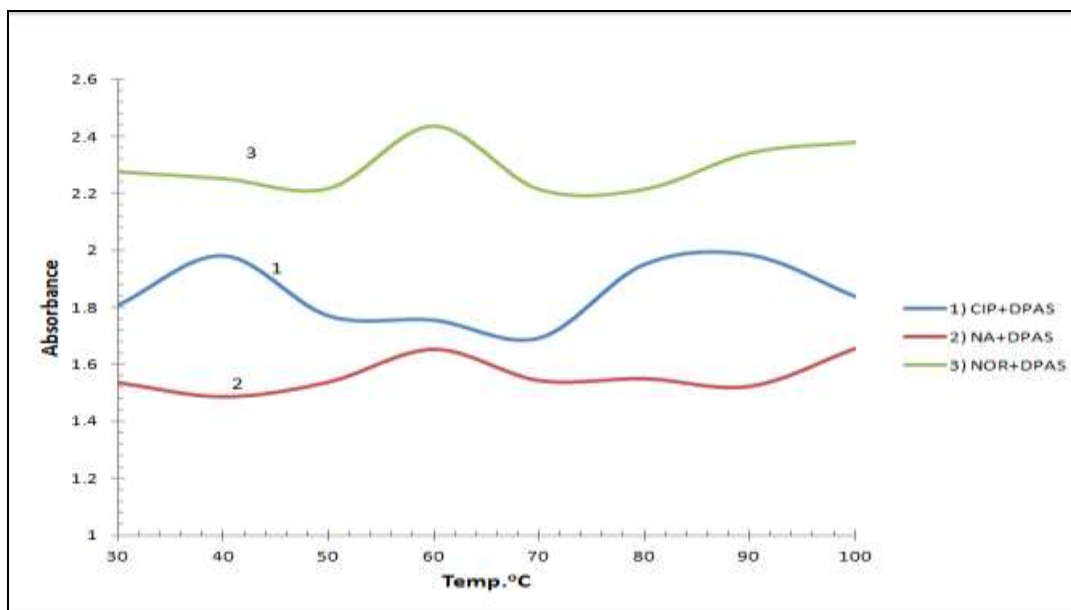
While the  $\lambda_{max}$  of the reaction products of the studied drugs with DPAS is shifted from 550, 545, 550 nm (violet form) Figure (1) to 280, 245, 285nm (brown form) for CIP, NA, and NOR respectively. Also it is obvious that DPAS has low intensity in the UV region compared to the drugs and the reaction under these conditions may take another way.



**Fig (4):** UV spectra of  $4 \times 10^{-5} \text{M}$  DPAS oxidant indicator as a blank (curve 1) and  $4 \times 10^{-5} \text{M}$  mixture of DPAS oxidant, with drug CIP, NA or NOR (curves 2-4) at normal temp and after 30 min.

**Effect of temperature:** On studying the effect of temperature at 30 to 100 °C region; it gives the results in Fig (5). The violet reaction products between the cited drugs and DPAS oxidant indicator are highly sensitive to temperature change. These forms were changed into brown forms at high temperatures within few minutes

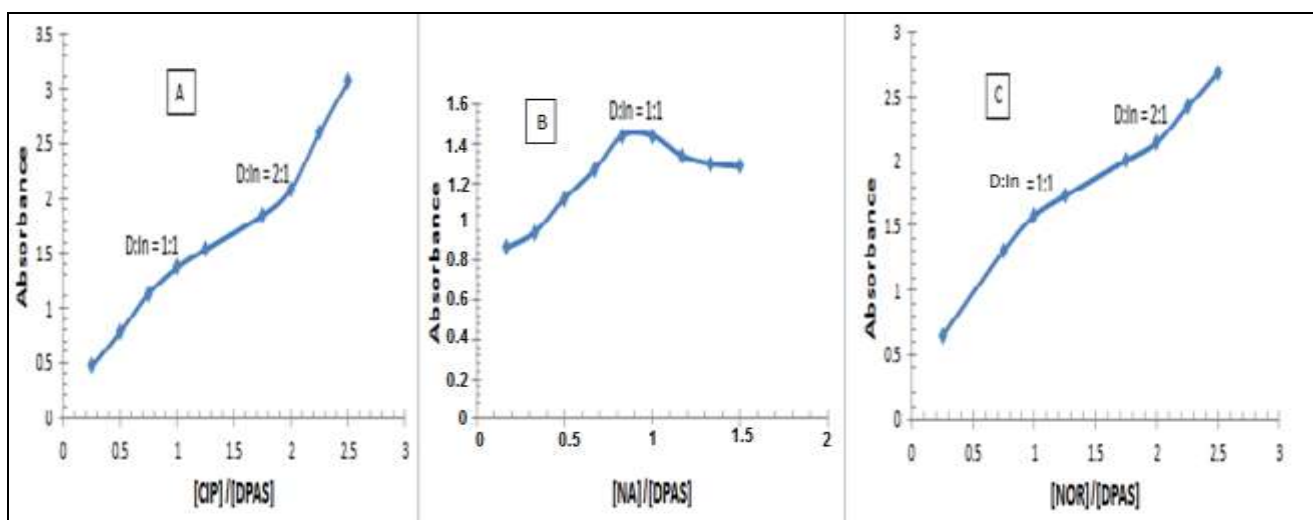
instead of one hour at normal temperature. These forms changed their internal structures into the new stable forms; which are detected in the UV region. Therefore it is possible to select 40°C for CIP and 60°C for both NA, NOR as suitable temperatures for micro-determination of the studied drugs in the UV region.



**Fig (5):** Effect of temp. (30-100°C) on spectra of  $4 \times 10^{-5} \text{M}$  mixture of DPAS oxidant with: 1) CIP (280 nm), 2) NA (245 nm), or 3) NOR (285 nm) after 30 min.

**Stoichiometric ratio:** The nature of the binding of indicator in its reduced form to the drugs in its oxidized form is determined by Job's method of

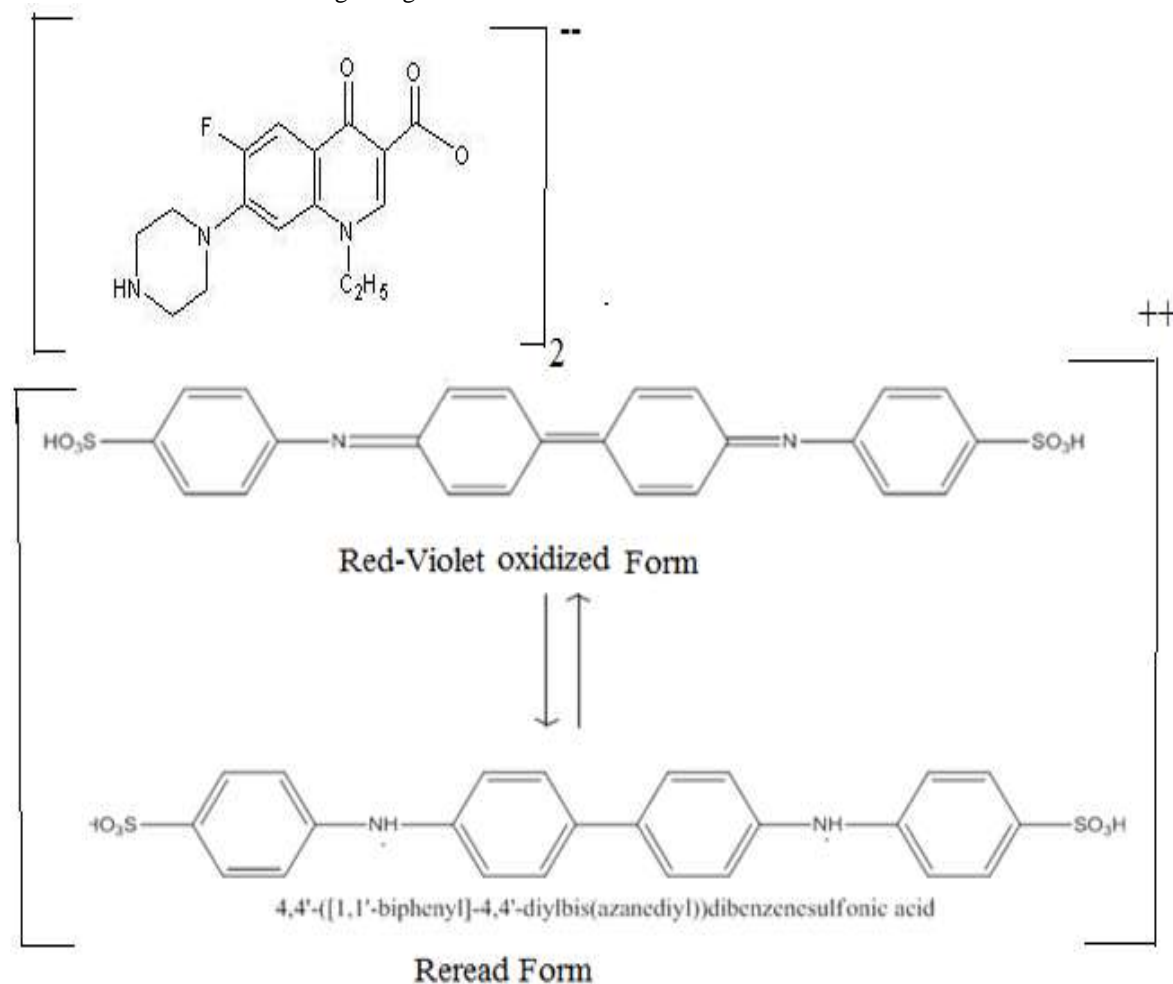
molar ratio method <sup>[36]</sup> using constant reagent concentration of DPAS indicator and variable concentrations of CIP, NA and NOR Fig (6).



**Fig (6):** Molar ratio of DPAS with different conc. of: A) CIP (280 nm, 40°C), B) NA (245 nm, 60°C), or C) NOR (285 nm, 60°C)

The results obtained indicate the formation of 1:1 and 2:1 ratios [drug]: [DPAS] ion-pairs through the electrostatic attraction between positive protonated DPAS indicator and drug negative anion.

Therefore; a proposal for the reaction mechanism taking Norfloxacin as an example is presented in Scheme 2.



**Scheme 2.** The proposed reaction mechanism of DPAS oxidant and NOR drug via formation of 1:1 ion-pair.

**Method validation:** Under the proper conditions described above, Beer's law was valid over the concentration range 19.3 – 154.3, 11.6 – 92.9, and 15.9– 127.7  $\mu\text{gml}^{-1}$  at violet form while 1.9 – 15.4, 2.3 – 9.3, and 1.6– 12.7  $\mu\text{gml}^{-1}$  at brown form for CIP, NA, and NOR using DPAS oxidant

respectively. Table (1) shows the different analytical parameters obtained such as slope, intercept, correlation coefficient, Sandell sensitivity, molar absorptivity ( $\epsilon$ ), standard deviation, and relative standard deviation, limit of quantification and limit of detection.

**Table 1:** Analytical parameters for spectrophotometric Determination of standard CIP, NA, and NOR drugs by the proposed DPAS methods

Reagent	DPAS		
	I. Violet Form		
DRUG	CIP	NA	NOR
Temp. ( $^{\circ}\text{C}$ )	Room temperature	Room temperature	Room temperature
$\lambda_{\text{max}}$ (nm)	550	545	550
Beer's law ( $\mu\text{g mL}^{-1}$ )	19.3- 154.3	11.6-92.9	15.9 - 127.7

<sup>a</sup> LOD ( $\mu\text{g mL}^{-1}$ )	4.4	3.97	6.03
<sup>b</sup> LOQ ( $\mu\text{g mL}^{-1}$ )	14.67	13.24	20.11
$R^2$	0.9999	0.9996	0.9988
<sup>c</sup> R.E. (Y)*	$y = -0.1363x + 2.5544$	$y = -0.0982x + 2.3897$	$y = 0.1044x + 1.5903$
<sup>d</sup> $\epsilon$ ( $\text{L mol}^{-1} \text{cm}^{-1}$ )	$0.1364 \times 10^4$	$0.985 \times 10^3$	$0.1039 \times 10^4$
<sup>e</sup> SD	0.33	0.24	0.31
<sup>f</sup> RSD %	0.56	0.63	0.49
<sup>g</sup> S.S ( $\mu\text{g cm}^{-2}$ )	$2.86 \times 10^{-1}$	$2.38 \times 10^{-1}$	$3.03 \times 10^{-1}$
Recovery %	99.98	99.86	100.58
<b>DRUG</b>	<b>II. Brown Form</b>		
	<b>CIP</b>	<b>NA</b>	<b>NOR</b>
Temp. ( $^{\circ}\text{C}$ )	40	60	60
$\lambda_{\text{max}}$ (nm)	280	245	285
Beer's law ( $\mu\text{g mL}^{-1}$ )	1.9 - 15.4	2.3 - 9.3	1.6 - 12.7
<sup>a</sup> LOD ( $\mu\text{g mL}^{-1}$ )	0.05	0.09	0.03
<sup>b</sup> LOQ ( $\mu\text{g mL}^{-1}$ )	0.18	0.31	0.09
$R^2$	0.9999	0.9998	0.9995
<sup>c</sup> R.E. (Y)*	$y = 0.3906x + 0.699$	$y = 0.2293x + 0.7183$	$y = 0.4427x + 0.509$
<sup>d</sup> $\epsilon$ ( $\text{L mol}^{-1} \text{cm}^{-1}$ )	$0.39098 \times 10^5$	$0.230 \times 10^5$	$0.4421 \times 10^5$
<sup>e</sup> SD	0.011	0.012	0.032
<sup>f</sup> RSD %	0.17	0.25	0.27
<sup>g</sup> S.S ( $\mu\text{g cm}^{-2}$ )	$9.87 \times 10^{-3}$	$1.01 \times 10^{-2}$	$7.22 \times 10^{-3}$
Recovery %	99.65	99.7	99.95

<sup>a</sup> The limit of detection., <sup>b</sup> The limit of quantification

<sup>c</sup> Regression equation  $A = a + b C$ , where C is the concentration in  $\mu\text{g/mL}$ .

<sup>d</sup> Molar absorptivity  $\text{L mol}^{-1} \text{cm}^{-1}$ ., <sup>e</sup> standard deviation., <sup>f</sup> Relative standard deviation.,

<sup>g</sup> Sandell sensitivity  $\mu\text{g cm}^{-2}$ .

It is obvious from Table (1) that; the accuracy and precision of the proposed methods are indicated by the small values of SD and RSD. The calculated values of Sandell sensitivity (S.S) and Molar absorptivity ( $\epsilon$ ) confirm the sensitivity of the methods. The linearity of calibration graphs are proved by the high values of the correlation coefficient ( $r$ ) and the small values of the  $y$ -intercepts of the regression equations. The limits of detection (LOD) and quantitation (LOQ) values are explaining the validation of the proposed method.

**Inter- and Intra- day study:** Table 2 presents the precision and validation of the proposed methods between the cited drugs and DPAS indicator at brown; are confirmed through the Inter- and Intra-day measurements, confirming adequate sample stability and method reliability. This is also confirmed for the five selected concentrations within the linearity range by the observed values of RSDs which are  $< 1\%$ .

**Table 2:** Within-day and In between- days spectrophotometric micro-determination of standard CIP, NA, and NOR drugs by the proposed DPAS method at UV region (Brown Form)

drug	[wt] taken ( $\mu\text{g mL}^{-1}$ )	[wt] found ( $\mu\text{g mL}^{-1}$ )		Recovery (%)		SD		RSD (%)	
		W-day	In- day	W-day	In- day	W-day <sup>a</sup>	In- day <sup>b</sup>	W-day <sup>a</sup>	In- day <sup>b</sup>
CIP	3.86	3.85	3.85	99.79	99.79	0.03	0.033	0.82	0.88
	4.82	4.79	4.79	99.33	99.32	0.038	0.035	0.79	0.72
	8.60	8.68	8.68	100.89	100.9	0.068	0.065	0.78	0.75



	10.61	10.67	10.65	100.57	100.38	0.05	0.045	0.47	0.42
	12.54	12.53	12.57	99.93	100.25	0.05	0.04	0.42	0.32
NA	2.90	2.91	2.92	100.2	100.2	0.028	0.028	0.95	0.95
	3.60	3.59	3.60	99.7	99.7	0.016	0.016	0.44	0.44
	4.99	4.97	4.98	99.5	99.5	0.029	0.029	0.59	0.59
	5.81	5.83	5.84	100.4	100.4	0.038	0.038	0.66	0.66
	6.97	6.97	6.98	100.04	100.04	0.056	0.056	0.81	0.81
NOR	2.71	2.75	2.74	101.3	100.95	0.024	0.014	0.88	0.51
	3.67	3.73	3.74	101.57	101.8	0.017	0.11	0.45	0.31
	5.59	5.6	5.58	100.2	99.85	0.043	0.038	0.76	0.68
	6.87	6.82	6.83	99.34	99.48	0.061	0.045	0.89	0.66
	10.38	10.42	10.41	100.4	100.31	0.054	0.055	0.52	0.52

<sup>a</sup> Mean values for five replicates experiments at each concentration level within 5 hours.

<sup>b</sup> Mean values for five replicates experiments at each concentration level at 5 days.

**Applications:** The proposed methods were successfully applied to determine CIP, NA, and NOR in their commercial tablets. The commonly used excipients and additives in the preparation of tablets did not to interfere in this analysis. The SD, % recoveries, and statistical analysis regarding the calculated student's t-test and variance ratio F-test [37] of the three drugs in their tablets compared with that of the official methods [6, 28, 38] are given in Table 3, The values did not exceed the theoretical tabulated values indicating that there is no significant difference between the proposed and the official methods regarding accuracy and precision.

### CONCLUSION

The proposed methods are simple, reliable, sensitive and efficient for routine analysis of this class of antibiotics in raw materials and in pharmaceutical dosage forms over a wide concentration range without interference from common excipients. In addition, the methods can be used spectrophotometrically at both UV and Visible regions. Moreover, it involves the advantage of the use of inexpensive instrument without losing accuracy. Therefore, these methods are useful for applications to the investigated drugs in bulk as well as in their tablets with high precision and good accuracy.

**Table3.** Spectrophotometric micro – determination of CIP, NA, and NOR drugs in pharmaceutical Formulations by proposed DPAS method and official method

Drug	Proposed Method Violet Form			Official Method			Proposed Method Brown Form			Official Method		
	Take n ( $\mu\text{g mL}^{-1}$ )	Foun d ( $\mu\text{g mL}^{-1}$ )	Recover y (%) <sup>a</sup>	Take n ( $\mu\text{g mL}^{-1}$ )	Foun d ( $\mu\text{g mL}^{-1}$ )	Recover y (%) <sup>a</sup>	Take n ( $\mu\text{g mL}^{-1}$ )	Foun d ( $\mu\text{g mL}^{-1}$ )	Recover y (%) <sup>a</sup>	Take n ( $\mu\text{g mL}^{-1}$ )	Foun d ( $\mu\text{g mL}^{-1}$ )	Recover y (%) <sup>a</sup>
CIP Ciprofar Tablet (500 mg / Tablet)	17.36	17.82	102.64	5	4.94	98.84	6.8	6.83	100.6	10	9.92	99.24
	86.81	86.27	99.38	10	10.12	101.24	8.72	8.81	101.04	20	19.92	99.61
	106.1	106.0	99.98	20	19.65	98.25	11.73	11.72	99.9	40	40.10	100.25
	121.5	122.0	100.46	40	40.62	101.54	12.65	12.62	99.73	60	59.87	99.78
	150.4	153.0	101.74	50	49.90	99.80	14.43	14.5	100.5	80	80.36	100.45
Mean±SD	100.84 ± 0.68			99.93 ± 1.44 [28]			100.35 ± 0.07			99.87 ± 0.49 [28]		
F-test	4.48 (6.39)**											
t-test	1.28 (2.45)**											
NA NalidramTa blet (500 mg / Tablet)	29.03	29.26	100.8	30	30.15	100.49	1.74	1.77	101.6	30	30.15	100.49
	40.64	40.11	98.7				3.02	2.98	98.7			
	46.45	46.71	100.6				4.06	4.03	99.2			
	52.25	51.89	99.03				5.81	5.82	100.2			
	81.28	81.65	100.45				7.55	7.56	100.2			
Mean±SD	99.92 ± 0.49			100.49 ± 0.46 [38]			99.98 ± 0.024			100.49 ± 0.46 [38]		
F-test	1.13 (6.39)**											
t-test	1.89 (2.31)**											
NOR Norbactin Tablet (400 mg / Tablet)	39.92	39.6	99.2	20	19.74	98.70	2.39	2.41	100.85	10	10.04	100.41
	55.88	55.8	99.9	40	40.76	101.91	3.99	4.03	100.96	20	19.88	99.38
	69.87	63.99	100.2	80	78.85	98.57	7.18	7.12	99.09	40	40.04	100.11
	71.85	72.8	101.3	120	120.0	100.07	8.78	8.8	100.21	60	60.27	100.45
	95.80	95.83	100.03	240	242.9	101.25	9.58	9.49	99.06	80	80.06	101.08
Mean±SD	100.13 ± 0.60			100.10 ± 1.49 [6]			99.99 ± 0.041			100.09 ± 0.43 [28]		
F-test	6.17 (6.39)**											
t-test	0.04 (2.57)**											



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