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A REVIEW OF SPECTROPHOTOMETRIC DETERMINATION OF ANTIBIOTIC NORFLOXACIN

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ABSTRACT: Norfloxacin is one of the 4-quinolone antibacterials and have many applications in veterinary and human medicine. It is extensively used antibiotic among fluoroquinolone antibiotics in both human and animal welfare. Therefore, the estimation and monitoring of norfloxacin in pharmaceutical preparations and effluents has become a topic of interest for researchers and industries. Existing methods require much time, expenses and sophisticated laboratory facilities. Most of the methods suggest the simultaneous determination of the drug along with the similar groups. There is a thirst among researchers for the development of simple, specific, sensitive and reliable methods suitable for routine laboratory facilities. Most of the methods are based on association of the drug either with metal complexes or reaction with pigments. The main objective of this paper is to review various existing methods for spectrophotometric determination of norfloxacin in order to explore the possibilities to overcome their drawbacks to develop new methods with enhanced simplicity, sensitivity, reproducibility, *etc.*

INTRODUCTION: Norfloxacin, [1-ethyl-6-fluoro -1,4-dihydro-4-oxo-7-(piperazin-1-yl) quinolin - 3 carboxylic acid] is an important broad-spectrum antimicrobial agent which belongs to fluoroquinolone family (**Fig. 1**). Its empirical formula is $C_{16}H_{18}FN_3O_3$, with molecular weight of 319.34g.mol^{-1}, and is white or pale yellow crystalline powder, very slightly soluble in water and slightly soluble in acetone and in ethanol [1, 2]. It is commonly used in the treatment of urinary tract infections, gonococcus urethritis and infectious diarrhea [3, 4].

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It is a synthetic antibiotic and used in the treatment of gastrointestinal or genitourinary infections. Its mechanism of action is through the inhibition of gyrase of the bacterial DNA thereby interfering with bacterial cell growth [5, 6, 7, 8]. Norfloxacin is active against both gram-negative and gram-positive micro organisms [9]. It is a widely used antibiotic medicine in India. The therapeutic importance of norfloxacin is also very common in Europe but is no longer used in United States [10].

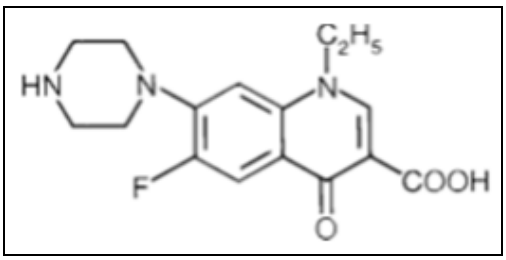

FIG. 1: CHEMICAL STRUCTURE OF NORFLOXACIN

Norfloxacin is generally well tolerated and its adverse effects are not very severe. Regardless of the fact that norfloxacin was introduced long back and many new fluoroquinolones have been introduced thereafter, it still holds a very important place in treatment of several infectious diseases [11].

Mechanism of action: The fluoroquinolone antibacterial norfloxacin is bactericidal in action [12]. Carboxylic and ketonic groups are responsible for the antibacterial activity, whereas substituents in positions 1 and 7 decide effectiveness and potency of the fluoroquinolone [13, 14]. Norfloxacin inhibits bacterial deoxyribonucleic acid (DNA) gyrase (topoisomerase II), an enzyme, which converts covalently closed circular DNA into negative supercoils [15, 16, 17, 18]. This DNA gyrase is able to introduce negative superhelical turns into duplex DNA.

It is believed that norfloxacin directly acts on DNA, producing a covalent attachment of DNA gyrase to DNA, which forms a complex that is inaccessible to the action of DNA polymerase; thus, leading to prevention of DNA synthesis and replication which ultimately results in rapid cell death [15, 19]. Thus, this unique mechanism of action accounts for the low rate of cross-resistance with other antimicrobial classes [20]. Quinolones similarly inhibit the *in vitro* activities of DNA topoisomerase IV by interfering with separation of replicated chromosomal DNA into respective daughter cells during cell division. This action is believed to be the primary target in gram-positive bacteria. At the molecular level, three specific events are attributed to norfloxacin [20, 21, 22]:

- **i.** Inhibition of ATP – dependent DNA supercoiling reaction catalyzed by DNA gyrase,
- **ii.** Inhibition of relaxation of supercoiled DNA, and
- **iii.** Promotion of the double – strand DNA breakage.

Analytical Methods for Determination of Norfloxacin: Various analytical methods have been reported for determination of norfloxacin either alone or in combined dosage form including; high performance liquid chromatographic methods [23, 24, 25, 26, 27, 28, 29], electro-analytical methods [30, 31, 32, 33], chemiluminescence [34, 35, 36, 37, 38, 39], spectrofluorimetry [40], and spectrophotometry [41, 42, 43]. Although HPLC has good analytical potential for determination of norfloxacin, but most laboratories lack in facilitating such sophisticated techniques [44]. Spectrophotometric methods are simpler in routine analyses of organics and metal ions in different matrices instead of using sophisticated and expensive instrumentations.

Reactions of fluoroquinolones with metal ions producing coloured complexes were utilized by many authors to develop methods for their determination. The complexation behaviour of fluoroquinolones was studied by many workers. For example, the complexation behaviour of norfloxacin with Fe(III) was studied by Lee *et al.*, [45]. The absorption, fluorescence and IR spectra of norfloxacin with Fe(III) were recorded. The formation constant of the 1:1 complexes was determined spectrophotometrically using Bjerum's method and scratched plots, the optimum pH for complexation was 3.8. The complexes were stable for 1 h. It was found Ni(II), Co (II), Mg (II) and Zn (II) did not interfere, but Cu (II) did [45].

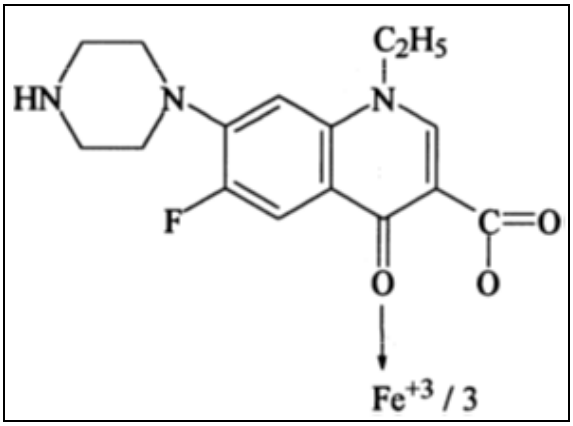

FIG. 2: COMPLEXATION BEHAVIOUR OF NORFLOXACIN WITH METAL IONS

Charge-transfer complexation between 4-quinolones as electron donors and certain π-acceptors formed the basis of several spectrophotometric methods.

Spectrophotometric Methods for Determination of Norfloxacin: Because of the presence of the carboxyl and carbonyl groups in neighbouring positions, the substance tends to act as a bidentate ligand. On the basis of this observation, various methods have been developed for

spectrophotometric determination of norfloxacin by the application of metal cations. Due to the piperazinyl nucleus norfloxacin has amine-like reactions, and forms ion-association complexes with charge transfer. A series of spectrophotometric methods were developed, in which norfloxacin given some complexes with coloring agents. Various spectrophotometric methods for the determination of norfloxacin and their analytical potential have been discussed below:

Amin *et al*., suggested some π-acceptors *i.e.* 2,3-dichloro-5,6-dicyano-p-benzoquinone (DDQ), 7, 7, 8,8-tetracyanoquinodimethane (TCNQ), p-chloranil (CL) and chloranilic acid (CLA) for spectrophotometric determination of norfloxacin (NRF). Norfloxacin reacts as a π-electron donor while DDQ, TCNQ, p-chloranil or chloranilic acid behave as π -acceptors. Their reaction of give highly colored complex species in non-aqueous medium. The reaction of DDQ with norfloxacin results in the formation of an intense orange-red product which exhibits an absorption maximum at 460 nm. The norfloxacin and TCNQ yielded an intense blue colour, causing characteristic long-wavelength absorption bands. The absorbance of the complex species formed between norfloxacin with DDQ, TCNQ, p-chloranil and chloroanilic acid were measured spectrophotometrically at 460, 843, 550 and 531 nm, respectively. The Beer's law for DDQ, TCNQ, p-chloranil and chloroanilic acid was obeyed in the ranges 20–400, 10-300, 10-230, and 20-250 µg.ml^{-1}. The values of molar absorptivity lie in the range of (1.1-8.8) x 10^3 l.mol^{-1}.cm^{-1} [46].

Supracene Violet 3B was another dye used for spectrophotometric determination of norfloxacin. This method was based on formation of norfloxacin-Supracene Violet 3B ion-association complex and its subsequent extraction into chloroform. The value of molar absorptivity of the complex was 5.88 x 10^3 l.mol^{-1}.cm^{-1} at λ_{max} 575 nm with Sandell's sensitivity of 0.054 µg.cm^{-2}. The calibration graph was linear over the range 5-40 µg.ml^{-1} of norfloxacin. The method offered the advantage that the drug can be determined individually in a multi-component mixture [47].

Most of the methods reported involve reaction of norfloxacin either with the metal cation or with a dye stuff for determination of the drug. However, El-Walily *et al.,* developed a method for determination of norfloxacin involving metal cation, dye and a surfactant. The method was based on the formation of a ternary complex between palladium (II), eosin and norfloxacin in the presence of methyl cellulose as surfactant. The ternary complex of norfloxacin-Pd(II)-eosin in presence of methyl cellulose exhibited an absorption maximum at 545 nm, with apparent molar absorptivity of 2.73 x 10^4 l.mol^{-1}.cm^{-1} and Sandell's sensitivity of 1.12 x 10^{-2} µg.cm^{-2} for norfloxacin. The method followed Beer's law within the concentration range 3-10 µg.ml^{-1}. The results obtained were compared with conventional methods and found to be suitable for routine laboratories due to its simplicity because it did not require an extraction procedure [48].

quantitative determination of norfloxacin through a sequential injection spectrophotometric method has been described by Suliman *et al.,* It was based on the complexation of norfloxacin with iron (III) in sulphuric acid media and spectrophotometric measurement of absorbance of the complex at 430 nm. Working range of the method was obtained to be 50-400 µg.ml^{-1} of norfloxacin. The technique has been used for flow injection quantitative estimation of the drug [49].

The reaction of norfloxacin with p-nitrophenol in aqueous medium forming a charge-transfer complex was the basis of another spectrophotometric method reported by Xuan *et al.*, The reaction requires temperature to be maintained between 30 to 60 ºC. The charge-transfer complex exhibited the maximum absorption at 407 nm. Method followed linearity within the concentration range of 0.3 – 16 µg.ml^{-1}. The molar absorptivity of the charge-transfer complex in terms of norfloxacin was found to be 1.1 x 10^4 l.mol^{-1}.cm^{-1} at its λ_{max}. The validity of the method for analysis of the drugs was examined by statistical analysis of the results. The (t) and (F) values indicated the method to be comparable in precision and accuracy with the official methods [50].

Some workers reported the pH-induced absorbance method for spectrophotometric determination of

norfloxacin. In this work, two spectrophotometric methods have been suggested in this paper. Both of the reactions have been performed in the presence of decarboxylated degradants. The first method applied the measurement of the pH-induced absorbance difference of the drug solution between 0.1 N HCl and 0.1 N NaOH at 280 nm. The working range of the method was found to be within 4-16 µg.ml^{-1} of norfloxacin. The second method was based on chelation of the intact drug with iron (II) in an acetate buffer solution at pH 5.7 which produced yellow-colored chelate with λ_{max} at 358 nm. The method measured norfloxacin in the concentration range of 16-64 µg.ml^{-1} [51].

In a charge transfer reaction between norfloxacin and 7,7,8,8-tetracyanoquinodimethane (TCNQ), norfloxacin acts as electron donor and TCNQ as electron acceptor has resulting a blue-colored charge transfer complex with maximum absorbance at 743 nm. This reaction was used for the spectrophotometric determination of norfloxacin. The Beer's law was obeyed in the concentration range 4.0–32 µg.ml^{-1} of norfloxacin. The apparent molar absorptivity of CT complex was 8.91 x 10^3 l.mol^{-1}.cm^{-1} at 743 nm. The statistical results obtained from the method were satisfactory and suitable for the determination of the drug without interference from common excipients such as starch and glucose or from common degradation products [52].

Rizk *et al.,* suggested a method in which norfloxacin was allowed to react with 3-methyl-2-benzothiazolinone hydrazone hydrochloride (MBTH) in the presence of cerium (IV) ammonium sulphate, which acts as an oxidant. The working range of the method was found to be in the concentration range of 20-100 µg.ml^{-1} of norfloxacin. The absorbance of the resulting complex formed was measured at 630 nm [53].

Amin described a method for the spectrophotometric determination of norfloxacin. This method was based on the formation of an ion-pair acetone soluble complex with anionic dye sudan III. Norfloxacin produced a bathochromic shift in the wavelength of sudan III from 510 to 567 nm. The effect of temperature was important in this reaction. At the ambient temperature (25 ± 2 ºC), complete color development require about 30 min. However, the time could be reduced to 5 min on raising the temperature to 50 ºC to obtain complete colour development. The value of molar absorptivity of the ion-pair complex in aqueous-acetone medium was found to be 2.61 x 104 l.mol^{-1}.cm^{-1} at the λ_{max} 567 nm. Beer's law was obeyed in the concentration range of 0.4-12.0 µg.ml^{-1} of norfloxacin. The method was found suitable for the determination of norfloxacin without interferences of excipients or degradation products in pharmaceutical formulations [54].

Determination of norfloxacin was performed by Hopkala *et al.,* The first, second, third and fourth order derivative spectra of the solutions of norfloxacin in the concentration range of 1.0-10.0 µg.ml^{-1} were recorded over the wavelength range 220-400 nm against 0.1 mol.l^{-1} HCl. The method comparably determined the drug by using peak-zero and peak-peak technique of measurement [55].

Rizk *et al.,* reported complexation of norfloxacin with Cu (II) in an aqueous medium was applied for UV-spectrophotometric determination of the drug in the concentration range of 15–80, ng.mL^{-1} [56].

In another method reported by Gowda *et al.,* the reaction of the norfloxacin with Brilliant Blue G (BBG) in sodium acetate-acetic acid (NaOAc-AcOH) buffer of pH 4.0 formed the basis of spectrophotometric determination of norfloxacin. The ion-association complex formed in this reaction was soluble in chloroform and its absorption maxima was found to be 614 nm for norfloxacin. The method obeyed Beer's law within the concentration range 0.4-8.0 mg ml^{-1}. The values of Molar absorptivity and Sandell's sensitivity for the method were 2.64 x 10^4 l.mol^{-1}.cm^{-1} and 12.07 ng.cm^{-2}, respectively. The results were compared statistically by the student t-test, and by the variance ratio F-test with those obtained by official/reported methods and found to be comparable [57].

For spectrophotometric determination of norfloxacin, El-Brashy *et al*., suggested application of two xanthene dyes, eosin Y and merbromin. Both methods are based on formation of binary complex between the drug and dye. These dyes form colored binary complexes with norfloxacin, which showed absorption maxima at 547 nm for

eosin Y and 545 nm for merbromin. Using eosin Y, the calibration graph was linear over the range 2–8 μg ml^{-1}. While in case of merbromin, the concentration range was 2–15 μg.ml^{-1}. The values of molar absorptivities of norfloxacin-eosin Y and norfloxacin-merbromin complexes were found to be 3.5347 × 10^4 and 1.2740 × 10^4 l.mol^{-1}.cm^{-1}, respectively. The methods were applicable without prior extraction of the colored binary complexes formed between norfloxacin and dyes and give results comparable to reference methods [58].

In another method, norfloxacin given a highly yellow colored complex instantaneously with the dye bromocresol green (BCG) in dichloromethane. The charge-transfer complex, formed in the reaction, has molar absorptivity of 2.27 x 10^{-4} l.mol^{-1}.cm^{-1} at its λ_{max} 412 nm. The method followed linearity over the concentration range 1-20 μg.ml^{-1} and has been successfully applied for spectrophotometric determination of norfloxacin with a mean percentage recovery of 99.99 ± 0.54 [59].

The reaction of norfloxacin with tetracyano ethylene (TCNE) in acetonitrile resulted in a charge-transfer (CT) complex with λ_{max}333 nm. The value of molar absorptivity of the complex was 2.64 x 10^{-4} l.mol^{-1}.cm^{-1}. The concentration range was 0.8-16 μg.ml^{-1} for norfloxacin using TCNE with mean percentage recoveries of 100.26 ± 0.68. TCNE behaved as π-electron acceptor and the presence of the F atom acting as an electron withdrawing group, the benzene ring in norfloxacin has lower electron density than the free terminal nitrogen atom in the piperazinyl moiety, so, norfloxacin behaved as π-electron donor. Hence, norfloxacin reacted with electron acceptor TCNE to form CT complex in presence of the solvent [59].

Rahman *et al.,* reported a kinetic spectro-photometric procedure for the determination of norfloxacin. The method is based on the oxidation of norfloxacin with alkaline potassium permanganate. The reaction was performed spectrophotometrically by measuring the rate of change of absorbance at 603 nm. The initial rate and fixed time (at 3 min) methods were utilized for constructing the calibration graphs to determine the concentration of the drug. The calibration graphs were found to be linear in the concentration ranges 2.0–20 μg.ml^{-1} and 1.0–20 μg.ml^{-1} using the initial rate and fixed time methods, respectively. There was no requirement of the elaborate treatment of the analyte and tedious extraction of the chromophore produced. The results were satisfactory in comparison with other methods [60].

Norfloxacin has been determined by the extractive spectrophotometric method which was based on the formation of blue-colored ion-associate between norfloxacin and cobalt (II) thiocyanate, at pH 2.5. The colored ion-associate was extracted into a mixture of organic solvents *i.e.* n-butanol and dichloromethane and absorbance was measured within the concentration range 20-240 μg.mL^{-1} of norfloxacin. Dichloromethane was mixed in n-butanol as an additive to enhance the extraction selectivity of n-butanol towards the ion-associate species. Molar absorptivity of the blue-colored ion-associate formed between norfloxacin and cobalt (II) thiocyanate was 8.21 x 10^2 l.mol^{-1}.cm^{-1} at its λ_{max} 623 nm. The method was successfully applied to determine norfloxacin over a wide concentration range without interference from common excipients, and the results were in good agreement with those obtained by the reference methods [61].

El-Brashy *et al.,* reported another method for the spectrophotometric determination of norfloxacin based on formation of ion-pair associate species. In this method, norfloxacin reacts with bismuth (III) tetraiodide forming a stable orange-red ion-pair associate which was instantaneously precipitated. The precipitate was filtered off and the residual unreacted bismuth (III) tetraiodide complex in the filtrate was analyzed spectrophotometrically within the concentration range 5-80μg.mL^{-1}. The value of molar absorptivity was found to be 1.92 x 10^3 l.mol^{-1}.cm^{-1} at its absorption maximum *i.e.* 453 nm. The filtered precipitate was dissolved in acetone and analyzed spectrophotometrically for which the method followed linearity in the concentration range 5-50 μg.ml^{-1}.

The orange-red ion-pair associate dissolved in acetone shown the molar absorptivity of 5.61 x 10^3 l.mol^{-1}.cm^{-1} at its absorption maximum *i.e.* 453 nm. The results were in good agreement with those obtained by the reference methods. The method was found to be advantageous that no extraction was needed to separate the ion-associates formed

avoiding the hazards of the organic solvents and being simpler and more convenient [62].

Spectrophotometric method using ammonium vanadate has been described for quantitative determination of norfloxacin. This method was based on a redox reaction between ammonium vanadate and the drug. In this reaction, ammonium vanadate was used to oxidise the drug norfloxacin in acidic medium, and vanadium (V) itself got reduced to vanadium (IV) by norfloxacin resulting in the development of a greenish blue colour measured at 766 nm. Beer's law limit was obtained in the concentration range of 10-40 $\mu g.ml^{-1}$ for norfloxacin. The method shown to be in good agreement with other methods and no interference was observed from commonly used excipients such as starch, talc, glucose, sucrose, magnesium stearate, sodium ascorbate, etc. [63].

Maheshwari *et al.,* reported an extraction-spectrophotometric method for norfloxacin by the application of hydrotropic solvents i.e. sodium benzoate and niacinamide which enhanced the aqueous solubility of the drug and gave an absorption maximum at 324 nm. The use of hydrotropic solvents was found to be successful in extracting norfloxacin from its dosage forms precluding the use of organic solvents. Since sodium benzoate and niacinamide do not absorb above 300 nm, but on extraction of norfloxacin into these hydrotropic agents, it exhibited the λ_{max} above 300 nm. This formed the basis of the spectrophotometric determination of norfloxacin in the presence of solublizing agents, sodium benzoate and niacinamide. However, the working range of the method is very high i.e. 5000–35000 $\mu g.ml^{-1}$ [64].

Askal *et al.,* reported the use of N-bromosuccinimide (NBS) as an analytical reagent for the spectrophotometric determination of norfloxacin. The procedure was based on the reaction involving two steps; first, the reaction of the norfloxacin with known excess amount of NBS and the second step involved the subsequent measurement of the excess unreacted NBS through its reaction with p-phenylenediamine (PDA) to give a violet colored product (NBS-PDA system) having absorption maximum at 530 nm. Beer's law was obeyed in the concentration range of 5-15 $\mu g.ml^{-1}$ of norfloxacin. The value of molar absorptivity of the method was found to be 1.74×10^4 $l.mol^{-1}.cm^{-1}$. The result obtained was comparable with those obtained by the official and other reported methods [65].

The measurement of absorbances of the red complexes formed between iron (III) and norfloxacin in acidic medium at 440 nm for the flow injection spectrophotometric determination of norfloxacin (NOR) has been reported by Al-Momani *et al.,* The intensity of the red-colored complex was found to be linearly related to the concentration of norfloxacin in the concentration range of 5 to 500 $\mu g.ml^{-1}$ of the drug. Relative standard deviation was less than 5.0% for the method. The statistical results obtained were compared with the reference methods and found to be satisfactory. There were no interferences from excipients or additives in any of the pharmaceutical samples analyzed [66].

Norfloxacin with ammonium reineckate, formed a pink-purple colored ion-association complex which was hardly soluble in water. The complex was highly soluble in the solvent dimethyl formamide (DMF).Norfloxacin reineckate presented maximum absorption at 524 nm, corresponding to the electronic transitions in the Cr(III) ion. The working concentration range of the method was 0.3-3.9 $\mu g.ml^{-1}$ of norfloxacin. Molar absorptivity of the ion-association complex was 3.4×10^4 $l.mol^{-1}.cm^{-1}$. Based on the precipitation reaction and on the UV-VIS spectral behaviour of the complex dissolved in DMF, the aim of the work was to establish simple and sensitive method for dosage of norfloxacin [67].

Another spectrophotometric method for the determination of norfloxacin was described using chemometric methods, such as classical least square (CLS), principal component regression (PCR), partial least square (PLS), and radial basis function-artificial neural network (RBF-ANN). Spectra were collected in the 190 - 400 nm range from a set of samples of norfloxacin in a Britton-Robinson buffer solution (pH=1.81). It was found that peak intensities were proportional to the concentration of norfloxacin. Beer's law was followed in the concentration range of 0.6-13.8 $mg.l^{-1}$ for norfloxacin [68].

A simple spectrophotometric method for the determination of norfloxacin (NF) based on measurement of the absorbance of NF at 291.6 nm in 0.1 N NaOH vs. 0.1 N HCl. The method did not require prior separation, and followed Beer's law was the concentration range of 2-20 $\mu g.ml^{-1}$ of NF. The lower limits of detection (LOD) and lower limits of quantification (LOQ) were 0.23 and 0.70 $\mu g.ml^{-1}$. The method was precise and satisfactory; with relative standard deviation of 1.5% (n=10). The recovery of the drug was 98.25 with relative error of 0.29 for NF. The results obtained were comparable the reference methods. The method was free from interferences of common excipients and additives [69].

In a UV-spectrophotometric method reported by Inamullah *et al.*, the quantitative determination of norfloxacin was carried out at 277 nm and its linearity range was found to be within 2-12 $\mu g.ml^{-1}$ of the drug by preparing the standard and sample solutions in 0.1 N hydrochloric acid. For the first order derivative spectrophotometric method, they determined the drug at 265 nm within the concentration range of 2-12 $\mu g.ml^{-1}$. The recovery was within standard limit of 99.23% to 101 % confirming accuracy of the methods. There was no any interference of excipients. The precision of the method has also been confirmed by the lower value of standard deviation [70].

Shinde *et al.,* proposed two spectrophotometric methods for estimation of norfloxacin in pharmaceutical dosage forms. The first method involved AUC (Area under Curve) in which the sampling wavelengths selected were between 272-282 nm over the concentration range of 2-12 $\mu g.ml^{-1}$ for Norfloxacin. The second method involved the Q-analysis (Absorbance Ratio Method) for which the sampling wavelengths selected were 277 nm over the concentration range of 2-12 $\mu g.ml^{-1}$ for norfloxacin. Percentage recovery was between 98-102% for the drug. The results of the statistical data were compared with the reference methods as per ICH guidelines and found to be suitable [71].

In the similar way, Pant *et al.,* described two spectrophotometric methods for the estimation of norfloxacin in pharmaceutical dosage forms. The first method involves determination using the Vierodt's Method (Simultaneous Equation Method); while the second method involves determination using the Multicomponent Mode. In both methods, the sampling wavelength selected was 273 nm over the concentration range of 2.5-20$\mu g.ml^{-1}$ for Norfloxacin. The methods shown lower values of standard deviation, RSD (<2%) and standard error. The RSD is also less than 2% as required by ICH guidelines. The % recovery of both methods were between 98% and 102% for the drug [72].

The ultraviolet (UV) and visible (VIS) spectrophotometric methods for the determination of norfloxacin in the pharmaceutical preparations have been described. In the UV method, norfloxacin exhibited an absorption maximum at 277 nm in 0.1 M hydrochloric acid medium. For the VIS spectrophotometric method, norfloxacin was allowed to react with chloranilic acid, which produced a purple colored complex with an absorption maximum at 520 nm. Acetonitrile was found to be the best solvent for chloranilic acid and norfloxacin both. The linearity of calibrations curves were observed over the concentration range of 2.0-7.0 $\mu g.ml^{-1}$ for the UV method and 90.0-120.0 μgml^{-1} for the VIS method. The relative standard deviation did not exceed 1.42% for Vis method.The visible spectrophotometric method was based on the reaction of norfloxacin as a π-electron donor with chloranilic acid as π-acceptor, resulting in a highly colored complex. The purple color of norfloxacin-chloranilic acid complex immediately reached its maximum intensity at room temperature and remained stable up to 30 min [73].

A cloud-point extraction molecular spectro photometric procedure has been described for dual detection of drug norfloxacin (NOR) and iron (III) ions in biological and pharmaceutical samples. In this method, the drug norfloxacin was allowed to react with Fe(III) ions in dilute acidic medium to form a colored hydrophobic (Fe(III)-NOR) complex. The complex was initially extracted into micelles of Triton X-114 as a mediated extractant followed the determination of both NOR and Fe(III) ions individually, by using spectro photometry at same absorption maximum of 432 nm. Method followed linearity within the concentration range of 2.5-120 μgml^{-1} for norfloxacin. The value of molar absorptivity was found to be 1.3×10^4 $l.mol^{-1}.cm^{-1}$ for norfloxacin [74].

TABLE 1: METHODS FOR THE SPECTROPHOTOMETRIC DETERMINATION OF NORFLOXACIN AND ITS APPLICATIONS

S. No.	Reagents for complexation	λ_{max} (nm)	Linear range (μg.ml^{-1})	Molar absorptivity (l.mol^{-1}.cm^{-1})	Applications
1.	2,3-dichloro-5,6-dicyano-p-benzoquinone (DDQ)	460	20–400	4.1 x 10^3	Pharmaceutical formulations
	7,7,8,8- tetracyanoquinodimethane (TCNQ)	843	10–300	8.8 x 10^3	
	p-Chloranil	550	10–230	1.2 x 10^3	
	Chloranilic acid	531	20–250	1.1 x 10^3	
2.	Supracene violet 3B	575	——	5.88 x 10^3	Pharmaceutical formulations
3.	Palladium (II) and Eosin	545	3–10	2.73 x 10^4	Tablet formulations
4.	Iron (III) in sulphuric acid medium	430	50–400	——	Pharmaceutical formulations
5.	p-Nitrophenol	407	——	1.1 x 10^4	Tablet formulations
6.	In 0.1 N HCl and 0.1 N NaOH	280	4-16	——	Pure and dosage forms
	Iron (II) in acetate buffer	358	16-64	——	
7.	7,7,8,8-Tetracyanoquinodimethane (TCNQ)	743	4.0–32	8.91 x 10^3	Pharmaceutical preparations
8.	3-methyl-2-benzothiazolinone hydrazone hydrochloride (MBTH) and Cerium ammonium sulphate	630	20–100	——	Pharmaceutical formulations
9.	Sudan III	567	0.4–12	2.61 x 10^4	Pharmaceutical formulations
10.	Derivative spectrophotometry	——	1–10	——	Tablet formulations
11.	Complexation with Copper (II)	——	0.015–0.080	——	Pure and dosage forms
12.	Brilliant blue G (BBG)	614	400–800	2.64 x 10^4	Pure and dosage forms
13.	Eosin Y	547	2–8	3.53 x 10^4	Tablet formulations and urine samples
	Merbromin	545	2–15	1.27 x 10^4	Tablet formulations and urine samples
14.	Bromocresol green (BCG) in dichloromethane	412	1–20	2.27 x 10^{-4}	Tablet formulations
	Tetracyanoethylene (TCNE) in acetonitrile	333	0.8–16	2.64 x 10^{-4}	Pharmaceutical formulations
15.	Kinetic spectrophotometry by oxidation with alkaline $KMnO_4$	603	2–20	——	Pharmaceutical preparations
16.	Cobalt (II) tetrathiocyanate	623	20–240	8.21 x 10^2	Tablet formulations
17.	Bismuth (III) tetraiodide	453	5–80	1.92 x 10^3	Tablet formulations and urine samples
	Bismuth (III) tetraiodide in acetone	453	5–50	5.61 x 10^3	Tablet formulations and urine samples
18.	Ammonium vanadate	766	10–40	——	Pharmaceutical formulations
19.	Hydrotropic solvents	324	5000–35000	——	Pharmaceutical formulations
20.	n-Bromosuccinimide	530	5-15	1.74 x 10^4	Tablets, eye drops, Ampoules
21.	Iron (III) in acidic medium	440	5-500	——	Tablets
22.	Ammonium reineckate reagent	524	0.3-3.9	3.4 x 10^4	Pharmaceutical formulations
23.	Chemometry with Britton-Robinson buffer sollution	190-400	0.6-13.8	——	Rabbit blood serum
24.	UV Spectrophotometry	291.6	2-20	——	Bulk powder and tablets
25.	UV Spectrophotometry in 0.1 N HCl	277	2-12	——	Tablets
26.	UV Spectrophotometry	277	2-12	——	Tablets

27.	UV Spectrophotometry: Vierodt's method and Multicomponent method	273	2.5-20	——	Tablets
28.	UV method in 0.1 M HCl medium	277	2-7	——	Tablets
	VIS method with Chloranilic acid	520	90-120	——	
29.	Dual detection method with Fe(III) and TritonX-114	432	2.5-120	1.3×10^4	Tablets

CONCLUSION: The methods reported for spectrophotometric determination of norfloxacin along with other fluoroquinolones offer good analytical potential. The results obtained from these methods were found to be comparable with the reference methods used for the determination of norfloxacin. Most of the methods can be applied for analysis and quality control of fluoroquinolone drugs in routine laboratories. However, emphasis must be given for the development of methods which may be more specific for the determination the drug so that the accuracy can be achieved to greater extent at minimal cost.

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