

SPECTROPHOTOMETRIC DETERMINATION OF SOME QUINOLONES ANTIBIOTICS FOLLOWING OXIDATION WITH CERIUM SULPHATE

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ABSTRACT

A simple, accurate and sensitive spectrophotometric method has been developed for the determination of ciprofloxacin, pefloxacin and sparfloxacin. The method was based on the oxidation of these drugs with cerium (IV) in the presence of perchloric acid and subsequent measurement of the excess Ce (IV) by its reaction with *p*-dimethylaminobenzaldehyde to give a brownish coloured product (λ_{max} at 470 nm). The decrease in the absorption intensity (ΔA) of the coloured product due to the presence of the drug was correlated with drug concentration in the sample solution. Optimum reaction conditions for the oxidation were established at 30°C for 5 minutes, and different variables affecting the reaction were carefully studied. Under the optimum conditions, linear relationships with good correlation coefficients of 0.9996, 1.000 and 0.9977 for ciprofloxacin, pefloxacin and sparfloxacin respectively were found between ΔA values and the concentrations of the drugs in a concentration range of 1.5706-6.2824; 15.8-47.4 and 1.86-7.44 $\mu\text{g mL}^{-1}$ for ciprofloxacin, pefloxacin and sparfloxacin respectively. Low limits of detection were obtained. The method was validated in terms of accuracy, precision and ruggedness. Reproducibility values did not exceed 2.0 % (coefficient of variation) for the drugs. The results obtained by the proposed method were comparable with those obtained by reference non-aqueous titration with advantages of speed, accuracy, precision and high sensitivity.

Keywords: Quinolone antibiotics, Spectrophotometric determination, Cerium sulphate, *p*-dimethylaminobenzaldehyde (DMAB), Oxidation.

INTRODUCTION

Quinolones are a class of antibiotics that have found wide use in therapy. The quinolones are very reactive against gram positive, gram negative and some anaerobic organisms. They are the latest class of antibacterial agents developed, the origin of which is accidental¹. Quinolones are a family of synthetic broad spectrum antibiotics. The majority of quinolones in clinical use belong to the subset of fluoroquinolones, which have a fluorine atom attached to the central ring system typically at the 6-position. The term 'quinolones' refers to the first generation of the potent and toxic synthetic chemotherapeutic agents derived from chloroquine used to treat serious complicated and life threatening bacterial infections. During the synthesis of chloroquine, an acidic substance was isolated from the mother liquor which had pronounced antibacterial activity. Its structure was characterized and it was named nalidixic acid. The first generation of the quinolones began with the introduction of nalidixic acid in 1962 for treatment of kidney infections in humans².

Large numbers of analogs and derivatives of the basic 4-quinolone structure have been synthesized over the past 35 years. Prototype quinolone antibacterials, such as nalidixic acid (naphthopyridine nucleus) and cinoxacin (crinoline nucleus) and piperimidic acid (pyridopyrimidine ring) differ in structure from the parent compound by the incorporation of an additional nitrogen atom into the quinoline nucleus. The early antibacterials have limited spectrum of activity, and newer compounds were sought to improve their antimicrobial spectrum. For the most part, the newer

drugs have a structure similar to the parent 4-quinolone first isolated from the synthesis of chloroquine and they possess halogen atom on the non-nitrogenated ring. A fluorine residue at position 6 is present on all of the newer agents.

The addition of piperazinyl ring at position 7 was found to extend the spectrum of activity leading to broad-spectrum fluoroquinolones. The fluoroquinolones are small molecules with weights between 300 and 500 Daltons. Many of these compounds are Zwitterions and exhibit different solubility characteristics with changes in pH. These agents are quite stable in both oral and parenteral dosage forms at or below 30°C. They have been shown to be stable in reconstituted serum and urine for several weeks, but they have chelation potential for transition metal ions, such as copper, lead, zinc and magnesium. The compounds are sensitive to strong light and should be protected from light for long-term storage to prevent loss of activity³.

The quinolones are divided into generations based on their antibacterial spectrum. The earlier generation agents have narrow spectrum than the later ones. There is no standard employed to determine which drug belongs to which generation. The only universal standard applied is the grouping of the non-fluorinated drugs found within this class (quinolones), within the first generation⁴. The fluoroquinolones are active against the enterobacteriaceae, *Pseudomonas* spp, *Stenotrophomonas* spp, Gram negative and Gram positive bacteria. They have little activity against anaerobic bacteria such as *Bacteroides fragilis* group (4 to 64 mg/L), *Peptostreptococcus* spp (0.5 to 4 mg/L).



Determinations of quinolones have been carried out in pharmaceuticals and biological samples using several methods. Assay of ciprofloxacin alone and along with some other quinolones has been done using techniques such as HPLC⁵⁻¹⁰, capillary electrophoresis^{11,12}, LC-MS^{13,14}, UV-spectrophotometric methods¹⁵⁻¹⁹ and ion-association complexes with aluminium/erythrosin²⁰, ternary complex formation with eosin and palladium²¹, luminescence²². Metal chelation has been used for several fluoroquinolones^{23,24}. Derivatization for spectrofluorimetric analysis has also been carried out using 4-chloro-7-nitrobenzofurazan in borate buffer pH 9.0²⁵. Sparfloxacin, on the other hand, has been assayed by such techniques as UV using bromothymol blue²⁶, non-aqueous titration²⁷ and microbiological method²⁸. ¹H and ¹⁹F NMR technique has also been reported for the analysis of pefloxacin, norfloxacin and ofloxacin²⁹.

Although many methods have been adopted for the analysis of these quinolones, some of the techniques describe the use of one reagent for the analysis of one quinolone at a time. Some of the procedures are quite laborious, not particularly sensitive, lack stability, involve solvent extraction and are carried out using highly sophisticated equipment which some of the time are beyond the reach of the third-world economies. None of the procedures currently reported utilize the Ce/DMAB system. The method described in this paper reports the accurate, precise and simple determination of three quinolones; ciprofloxacin, pefloxacin and sparfloxacin using excess cerium sulphate as oxidising agent and then determining the excess unreacted Ce using *p*-dimethylaminobenzaldehyde.

MATERIALS AND METHODS

Equipment

Lambda 25 UV/VIS Spectrophotometer, Mettler AE 160 balance, thermostated water bath, Pye UNICAM colorimeter (Model SP8-400, Cambridge), test tubes, micro-pipette (Pipetman – Gilson, France).

Materials and Reagents

Para-dimethylaminobenzaldehyde (DMAB) was obtained from BDH and recrystallized from methanol. Ciprofloxacin, Pefloxacin and Sparfloxacin secondary reference were obtained from their respective tablets and their identities and purities were assessed by melting point determinations and non-aqueous titration. Methanol, perchloric acid, sodium hydroxide, ethanol, acetic acid, Ceric ammonium sulphate, sulphuric acid, phosphoric acid, hydrochloric acid, acetonitrile, *n*-propanol are all analytical reagent grades from BDH, Poole, England.

Preparation of stock solutions:

Ceric ammonium sulphate, 0.15% (w/v in 4 M perchloric acid) and 0.2% (w/v in methanol) of *p*-dimethylaminobenzaldehyde (DMAB) were freshly prepared daily.

Respective drug stock solutions were prepared by dissolving weighed quantities in methanol to give molar concentrations of 2.37×10^{-3} M for ciprofloxacin, pefloxacin and sparfloxacin.

Tablets analysed in this study:

Ciprofloxacin tablets: Vitapro (Global remedies Ltd), Cenox (Microlabs Ltd, India), Ciprotab (V.S International pvt Ltd, India), Ciprocil (Glenmark laboratories pvt Ltd, India).

Pefloxacin tablets: Peflotab (V.S International pvt.ltd.), Pinox (Microlabs Ltd, India), Peflomed (Bharat parenterals Ltd, India)

Sparfloxacin tablets: Sparx (Wockhardt Ltd, India), Sparmax (Microlabs Ltd, India), Sloxin 200 (Naxpar lab pvt Ltd, India).

Preparation of tablet samples for analysis:

Twenty tablets of each drug were weighed and finely powdered. The stock standard solutions were prepared by dissolving an accurately weighed amount of the powdered tablet contents equivalent to the active ingredient which was transferred into a 100 mL calibrated flask and dissolved in about 50 mL of methanol. The contents of the flask were swirled and then completed to the volume with methanol. The dispersions were thereafter filtered and used for sample analysis.

Methods

General procedure for the reaction of ciprofloxacin, pefloxacin and sparfloxacin with Cerium (IV) and DMAB

One millilitre of the drug stock solution was measured into a 5 mL volumetric flask, one millilitre of ceric ammonium suitable [0.15 % w/v perchloric acid] was also added, it was mixed well using a vortex mixer and allowed to stand for 30 minutes at room temperature [25 ± 5 °C], then 1 mL of DMAB reagent solution was added and allowed to stand for 1 min. The solution was then completed to the mark with distilled water. The decrease in absorbance was measured at 470 nm against blank treated, but without each drug.

Selection of analytical wavelength

One mL aliquot of the drug stock solution was added into test tube and 1 mL of ceric ammonium sulphate [0.15%] was also added, it was mixed and allowed to stand for 30 minutes at room temperature, then DMAB [0.2%] reagent solution was added and allowed to stand for 1 min before completing with 2 mL of H₂O. This solution is then mixed and the spectra were recorded on a UV-VIS spectrophotometer using the range 200-700 nm against blank prepared with the mixture of ceric ammonium sulphate [0.15% and DMAB (0.2%)]. The wavelength at which there is highest decrease in absorbance between cerium and a mixture of cerium, drug and DMAB was then selected as analytical wavelength.



Determination of molar ratio of the reactions for drugs (Ciprofloxacin, Pefloxacin, Sparfloxacin) with Ce (IV)

The mole ratio method was employed. 1 mL aliquots of drug solution (0.00237M) were transferred into 5 mL calibrated flasks. To each flask, 0, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1 mL aliquots of Ce (IV) solution (0.00237M) were added and the reactions were allowed to proceed for 30 minutes at room temperature ($25 \pm 5^\circ\text{C}$). One millilitre of DMAB solution (0.2%w/v) was added to each flask and the reaction mixtures were completed to volume with water. The decrease in absorbance was measured at 470 nm against reagent blanks treated similarly, except that the drugs were omitted.

Determination of molar ratio between Ce (IV) and DMAB.

The Job's method of continuous variation [30] was employed. Equimolar solutions (2.37×10^{-3} M) of Ce (IV) and DMAB were prepared. Series of 5 mL portions of the master solutions of ceric ammonium sulphate and DMAB were made up comprising different proportions (0:1, 0.2:0.8, 0.3:0.7, 0.4:0.6, 0.5:0.5, 0.6:0.4, 0.7:0.3, 0.8:0.2, 1:0) in 5 mL volumetric flask. The reactions were allowed to proceed at room temperature ($25 \pm 5^\circ\text{C}$). The reaction mixtures were completed to volume with water and absorbance was measured at 470 nm against reagent blanks prepared without Ce (IV).

Optimization studies of reaction variables

Effect of temperature and time on oxidation reaction

The effect of temperature was studied at five levels of 30°C , 50°C , 60°C , 70°C and 80°C each maintained for 5 and 20 minutes. 1 mL of drug stock solution was added to Ce (IV) and DMAB of equimolar ratio. The solution was then cooled in an ice bath and the absorbance was taken at 470 nm. The effect of time was studied at different levels of 0, 2, 5, 10, 15, 20, 25 and 30 minutes each maintained at the optimized temperature before the absorbance is taken.

Effect of other reaction variables

The effect of acid type, acid concentration, cerium (IV) concentration, DMAB concentration and the diluting solvents were studied and optimized using the general procedure.

Validation studies

Linearity of response for each drug (Ciprofloxacin, Pefloxacin, and Sparfloxacin) for the reaction with cerium (IV) using the range of 0-50 $\mu\text{g/mL}$ from the drug stock solution in methanol was prepared. For ciprofloxacin and pefloxacin, the absorbance difference at 470 nm in the presence of the drug and cerium and that between DMAB and cerium was recorded. While the absorbance difference between cerium alone and cerium with sparfloxacin was recorded. All absorbance readings after the reaction interval at the optimized temperature and the optimized time were recorded at 470 nm. Linear regression analysis and ANOVA were used to select the

dynamic working range. Replicate samples were prepared for the calibration. The regression line equation, correlation coefficient and limit of detection were obtained from the calibration curve.

The recoveries of the concentrations of each drug from laboratory samples were determined on a three day assessment as a measure of accuracy of the new method. The accuracy of the method was evaluated by the standard addition method at three different concentrations levels.

The precision of the new method was assessed using replicate samples at different concentration levels within the range of 1.5706 - 6.2824 $\mu\text{g/mL}$ for ciprofloxacin, 15.8 - 47.4 $\mu\text{g/mL}$ for pefloxacin and 1.86 - 9.30 $\mu\text{g/mL}$ for sparfloxacin. Each concentration of the drug (ciprofloxacin and pefloxacin) was reacted with cerium and DMAB and with Cerium alone (sparfloxacin) using the optimized conditions for each drug, and absorbance readings were taken at 470 nm. The concentrations of the drugs were estimated from the regression line equation. The precision was thereafter estimated with percent relative standard deviation (% coefficient of variation). Three day assessment was done.

Dosage forms Analysis

Three different brands of each of ciprofloxacin, pefloxacin and sparfloxacin tablets were evaluated for their content of active ingredient using the new method. Weight uniformity test was carried out on each brand of the tablets. Twenty (20) tablets were crushed and powdered. An amount of the powder equivalent to 0.00237 M of ciprofloxacin, pefloxacin and sparfloxacin respectively was dissolved in 10 mL of methanol each in separate sample vials. The dispersions were allowed to stay for 1 hour with intermittent swirling and then filtered. 1 mL of the resulting solution was reacted with 0.8 mL, 0.7 mL of cerium (IV) and 0.2 mL, 0.3 mL of DMAB for ciprofloxacin and pefloxacin respectively while 0.5 mL of sparfloxacin was reacted with 0.5 mL cerium (IV) at 30° for 5 minutes. The absorbance was measured at 470 nm. The concentration corresponding to the measured absorbance was determined using the regression line equation. Six replicates were determined for each of ciprofloxacin, pefloxacin and sparfloxacin tablets.

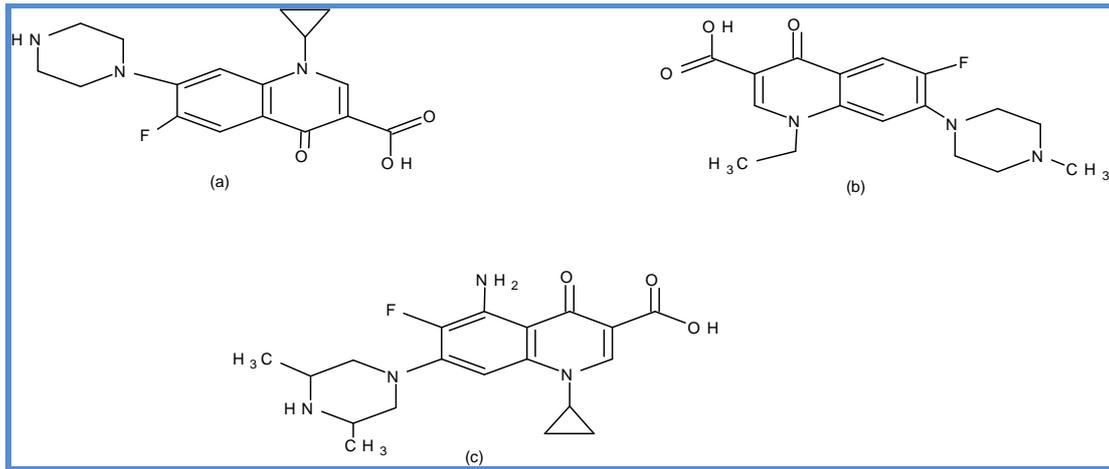
Non-aqueous titration was used as reference procedure for comparison with the results of the new method.

Interference studies

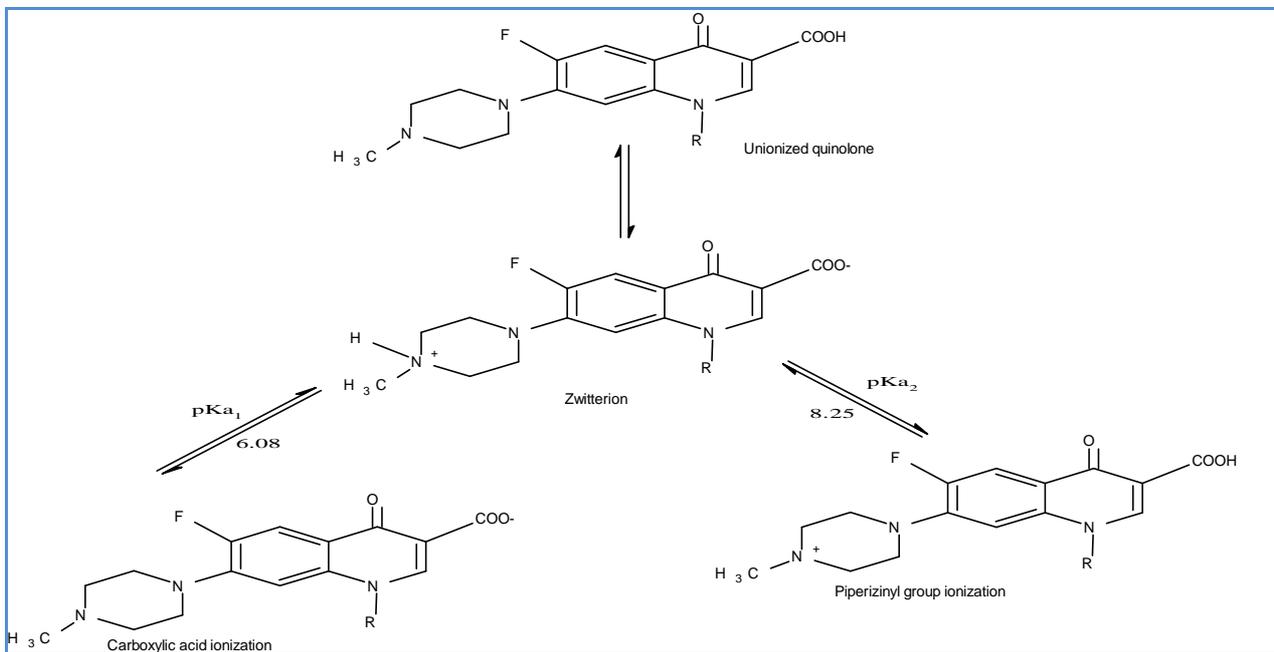
Samples were prepared by mixing 0.5, 0.7 and 0.8 mL of sparfloxacin, pefloxacin, ciprofloxacin reference solution with 10 mg quantities of common excipients such as lactose, starch, talc, gelatin and magnesium stearate and allowed to stay for 10 minutes. Cerium solution and DMAB were added as done previously. The analyses of these laboratory prepared samples were carried out using the general procedure and the recoveries were evaluated.



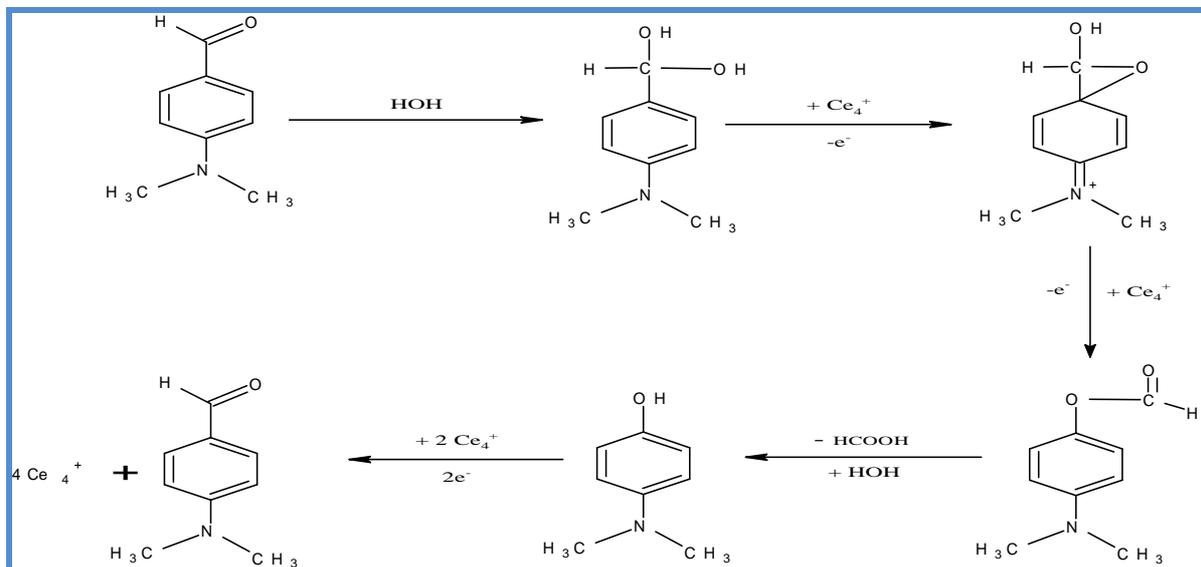
Figure 1: Structures of the Quinolone antibiotics studied; Ciprofloxacin (a), Pefloxacin (b) and Sparfloxacin (c)



Scheme 1: Ionization of quinolones showing groups that can be oxidized



Scheme 2: Proposed reaction mechanism between DMAB and Ce (IV) in the analysis of ciprofloxacin and pefloxacin



RESULTS AND DISCUSSION

The proposed procedures involve the oxidation of the quinolones with known excess of cerium (IV) to generate a brown coloured solution and then measuring the excess unreacted Ce (IV) through its reaction with DMAB (for ciprofloxacin and pefloxacin) and directly (sparfloxacin). The structures of the quinolones are presented in Fig 1.

All the three quinolones possess carboxylic acid moieties and the piperinyl group which have the potentials of ionizing and reacting as acids (Scheme 1). The respective ionization will reduce Ce (IV) to Ce (III) and the drugs in turn get oxidised. The amount of cerium left unreacted following the oxidation process was used as a quantitative measure of the amount of quinolones present in the sample matrix. The addition of DMAB to Ce (IV) resulted in the formation of reddish brown coloured product which absorbed maximally at 470 nm. The decrease in absorption intensity (ΔA) at 470 nm caused by the presence of the drug was proportional to the amount of drug in the sample. Both the drugs and DMAB have no significant light absorption at this wavelength. Thus 470 nm was adopted as the analytical wavelength. The absorption spectra of ciprofloxacin, DMAB and Ce (IV) are overlaid on each other and presented in Fig. 2 to illustrate the pattern of reaction of the quinolones measured spectrophotometrically.

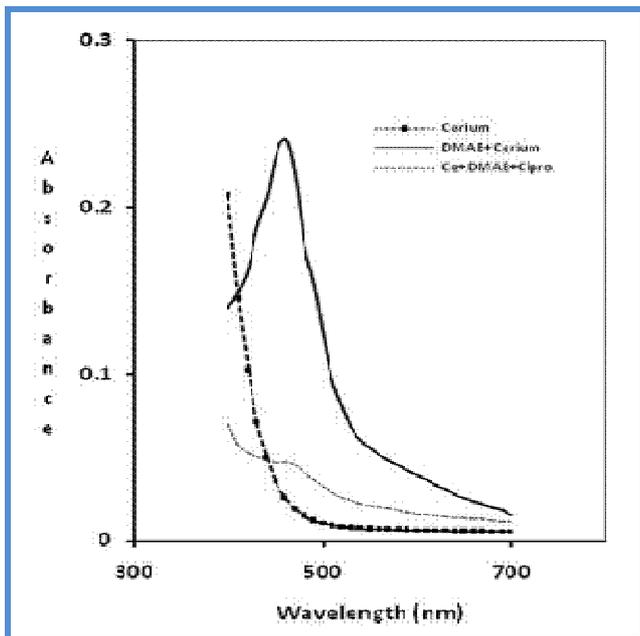


Figure 2: Overlaid Absorption spectra of ciprofloxacin, DMAB and Cerium (IV)

Optimization of reaction variables

Cerium (IV) was added in excess in all the reaction procedures and the amount left unreacted was estimated. The effect of temperature and time on the reaction was studied using the method of steepest ascent at temperature levels of 30°C, 50°C, 60°C, 70°C and 80°C for 5 and 20 minutes. The result obtained is presented in Fig. 3. All the three investigated drugs produced optimal

results at room temperature. As evident from the figure, the absorbance intensity decreased considerably and drastically with increase in temperature for ciprofloxacin, pefloxacin and sparfloxacin. Thus high temperature seems to be causing instability in the reaction medium. Thus 30 °C was selected as optimum temperature for this procedure.

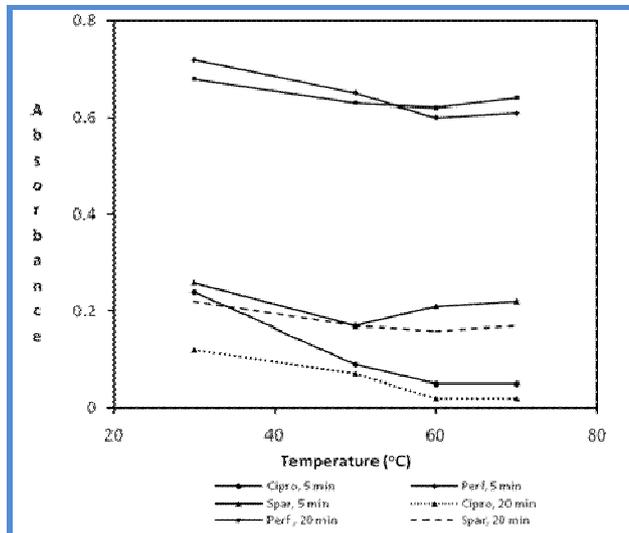


Figure 3: Optimization of reaction temperature

The time required for the completion of the oxidation of the quinolones with Ce (IV) was thereafter studied at time levels of 0, 2, 5, 10, 15, 20, 25 and 30 minutes by stopping the reaction with the addition of DMAB (ciprofloxacin and pefloxacin) and the diluting solvent or the diluting solvent directly (sparfloxacin). The result is presented in Fig. 4.

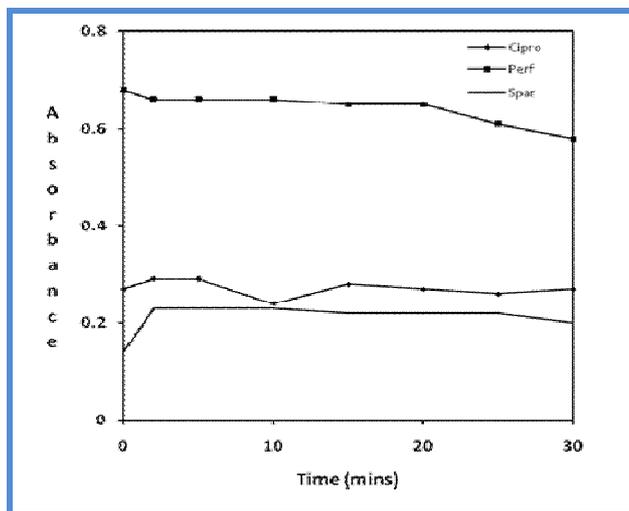


Figure 4: Optimization of reaction time

For sparfloxacin, the highest absorbance difference was obtained at 5 minutes and a plateau occurred till 10 minutes with gradual decrease in absorbance intensity for the remaining period. For pefloxacin, a slight decrease in absorbance was obtained from 0 minutes to 2 minutes and the value thereafter remained constant till 20 minutes. For ciprofloxacin, the absorbance intensity also attained a plateau at 5 minutes. Thus the optimum time

was set at 5 minutes at room temperature for the three quinolones, as significant loss of accuracy will not be observed at this time level.

In order to determine the most suitable acid for the reaction, different acids (sulphuric acid, phosphoric acid, hydrochloric acid and perchloric acid) were used in preparing the cerium sulphate solution and their effects were assessed at the optimized time (5 min) and the optimized temperature (30°C) at 470 nm. The results revealed that the reaction of quinolone drugs with Ce (IV) proceeded quantitatively only in the presence of perchloric acid, as the highest (ΔA) absorbance values were obtained when perchloric acid was used. The highest absorbance with Ce (IV) in perchloric acid can be attributed to the highest oxidation potential of Ce (IV) in perchloric acid ($E_0 = 1.75V$) as compared to that of Ce (IV) in 4 M sulphuric acid ($E_0 = 1.44V$), hydrochloric acid ($E_0 = 1.28V$)³¹. Moreover the oxidation of organic compounds by Ce (IV) in perchloric acid overcomes both the slowness of the oxidation process and the inexact stoichiometry encountered in sulphuric acid. The solutions of Ce (IV) in hydrochloric acid are unstable owing to the oxidation of chloride ions to chlorine gas, which is also a powerful oxidising agent³². The oxidation reaction was conducted in acid medium to avoid precipitation of hydrated ceric oxide ($CeO_2 \cdot H_2O$).

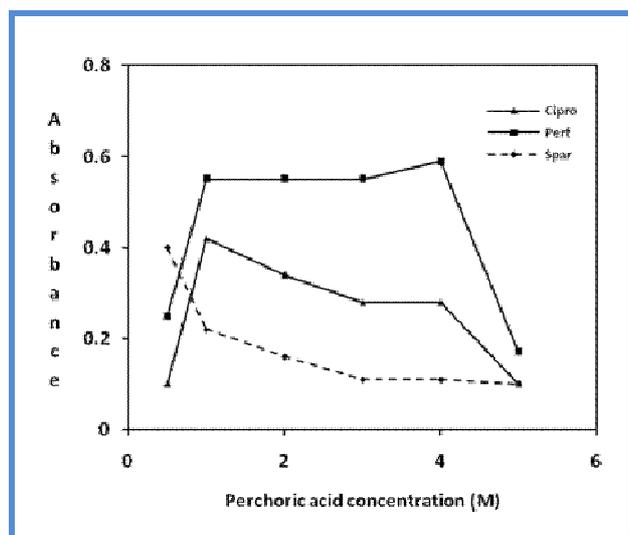


Figure 5: Effect of perchloric acid concentration on the reaction of quinolones with Ce (IV)

The effect of perchloric acid concentration on the reaction of the quinolones with studied drugs was investigated using different acid concentration in the range of 0.5 M – 6 M. The result for ciprofloxacin showed that the absorbance (ΔA) increased as the acid concentration increased to 1 M and the absorbance started decreasing from 2 M. Therefore, 1M perchloric acid was selected as perchloric acid concentration for ciprofloxacin analysis while for pefloxacin, the absorbance increased with increase in acid concentration till 4 M before decreasing. Therefore 4 M perchloric has the highest absorbance for pefloxacin. For sparfloxacin, the absorbance decreased with increase in acid

concentration till 1 M and decreased thereafter with increase in acid concentration. The result is presented in Fig. 5.

The effect of Ce (IV) reagent concentration on this reaction was carried out by reacting 0.2 mL and 0.3 mL DMAB with 0.8 mL and 0.7 mL of cerium (IV) in the range of 0.05 - 0.50 % w/v with 1 mL of ciprofloxacin, pefloxacin respectively and 0.5 mL Ce (IV) with 0.5 mL Sparfloxacin. The result is presented in Fig. 6. It can be observed that for the drugs, the absorbance increased with increase in the concentration of the reagent. The highest absorbance for ciprofloxacin, pefloxacin and sparfloxacin was obtained when concentrations of 0.30% w/v, 0.40%w/v and 0.25% of Cerium (IV) were used respectively.

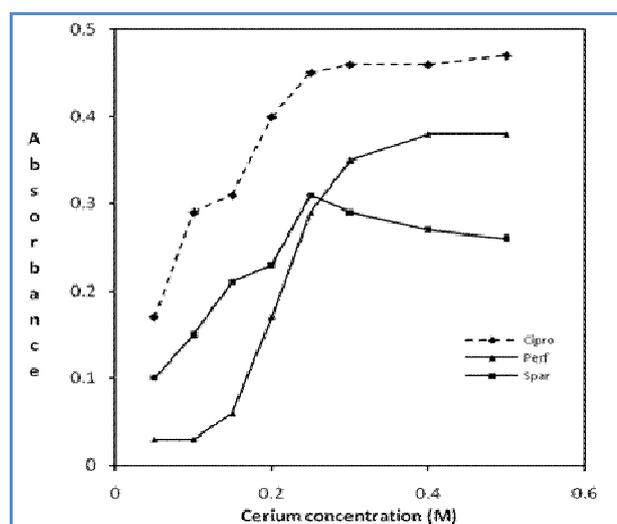


Figure 6: Effect of Ce (IV) concentration on the reaction with quinolones

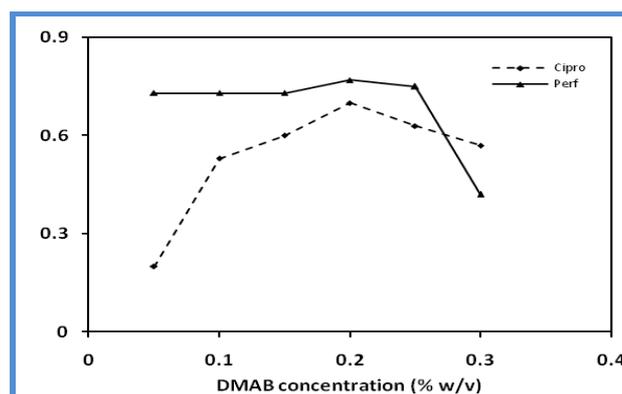


Figure 7: Effect of DMAB concentration on the reaction of Ciprofloxacin and pefloxacin with Ce (IV)

The effect of DMAB concentration on the reaction of ciprofloxacin and pefloxacin was carried out by reacting 0.2 mL and 0.3 mL of different concentration of DMAB in the range of 0.05% - 0.40% w/v with 0.8 mL and 0.7 mL of cerium (IV) with 1 mL of each drug for ciprofloxacin and pefloxacin respectively. It was observed that for the drugs, the highest absorbance was obtained at a

concentration of 0.20 % w/v DMAB. The result is presented in Fig. 7.

DMAB reagent was observed to be unsuitable for the measurement of the excess unreacted Ce (IV) for sparfloxacin. Upon adding DMAB after the reaction interval, a completely colourless solution was obtained which made it difficult to assess the excess unreacted Ce (IV). As shown in Fig. 1, the presence of additional amino group at the 5th position may be causing this interference. Since the reaction is being carried out in an acidic medium, the possibility of the formation of a Schiff base with DMAB may not be ruled out and thus the colourless solution may signify the formation of this new adduct. We have previously reported the formation of a Schiff base between DMAB and hydralazine in a simple acid solution³³. Thus for sparfloxacin, the excess unreacted Ce (IV) was estimated directly and correlated with the drug concentration without recourse to the use of DMAB reagent.

Different solvents (water, methanol, ethanol, acetonitrile, *n*-propanol) were investigated to select the proper solvent for dilution. The optimum reagent concentration, DMAB concentration, temperature, and time for each drug were kept constant. The highest ΔA value were obtained when water was used as solvent for ciprofloxacin and pefloxacin while for sparfloxacin, *n*-propanol has the highest absorbance value as presented in Fig. 8.

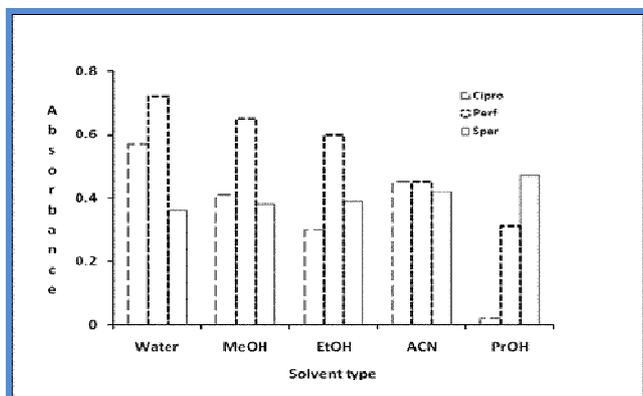


Figure 8: Optimization of diluting solvent for the oxidimetric procedure

Table 1: Quantitative parameters for the assay of the three quinolones using this oxidation procedure

Compound	Range ($\mu\text{g/ml}$)	Intercept (a)	Slope (b)	Correlation coefficient (r)	Molar absorptivity ($\text{Mol}^{-1}\text{cm}^2$)	LOD ($\mu\text{g/ml}$)	LOQ ($\mu\text{g/ml}$)
Ciprofloxacin	1.57-6.28	-0.01	0.07132	0.9996	22644	0.4627	1.40213
Pefloxacin	15.8-47.4	0.125	0.00743	0.99983	35572.7	2.048	6.2068
Sparfloxacin	1.86-9.3	-0.001	0.0117	0.99977	4645	1.3353	4.0464

Table 4: Assessment of the Method ruggedness for the new spectrophotometric determination of quinolones

Compound	Recovery (% \pm S.D.) ^a		
	Day 1	Day 2	Day 3
Ciprofloxacin	98.81 \pm 1.38	102.37 \pm 1.05	99.35 \pm 2.01
Pefloxacin	99.03 \pm 1.57	100.47 \pm 1.33	100.98 \pm 1.36
Sparfloxacin	98.94 \pm 2.92	98 \pm 3.21	99.45 \pm 1.67

^a values are means of three determinations \pm S.D.

Method Validation

Under the above mentioned optimized conditions, the calibration graphs correlating the decrease in the absorption intensity (ΔA) with the corresponding concentration of the drug were constructed. Positive correlations of absorbance on concentration and excellent correlation coefficients (r) of 0.9996, 0.9998 and 0.9997 for ciprofloxacin, pefloxacin and sparfloxacin were obtained respectively. In all cases Beer's law plots were linear with small intercept (-0.001 to -0.125) and high slope. For all the three drugs, an advantage of the calibration range is the micro levels which can be detected which offer some great advantage over previously reported spectrophotometric methods for the determination of these quinolones. The sensitivity was indicated by the fairly high value of the molar absorptivities for the three compounds. This sensitivity is higher compared to the low sensitivity recorded by previous methods based on direct oxidation with cerium and after determination of excess cerium using reagents such as indigo carmine or methyl orange.

The limits of detection and quantitation for the assay of the three quinolones were assessed from the calibration data. Limit of detection is the lowest amount of analyte in a sample that can be detected. It is determined using the expressions;

$$LOD = \frac{3.3 \sigma}{s} \quad \text{and} \quad LOQ = \frac{10 \sigma}{s}$$

Where σ is the standard deviation of the blank signals and s is the slope of the calibration graph³⁴.

The various quantitative parameters for the assay of the three quinolones using this oxidation procedure are presented in Table 1.

Table 2: Intra-day accuracy of the proposed spectrophotometric method

Drug	Amount taken ($\mu\text{g mL}^{-1}$)	Amount found ($\mu\text{g mL}^{-1}$)	Recovery (%) ^a	RSD (%) ^b	Relative error
Ciprofloxacin	1.5706	1.521	96.87	2.35	0.049
	4.7118	4.697	99.69	1.485	0.0148
	6.2824	6.275	99.88	0.97	0.0074
Pefloxacin	15.8	15.348	97.14	1.65	0.452
	31.6	31.271	98.96	1.81	0.324
	47.4	47.869	100.99	1.6	0.469
Sparfloxacin	1.86	1.822	97.96	2.31	0.038
	5.58	5.354	95.95	1.04	0.226
	7.44	7.66	102.92	0.96	0.22

^a Average of six determinations; ^b RSD, relative standard deviation

Table 3: Inter-day accuracy of the proposed spectrophotometric method

Drug	Amount taken ($\mu\text{g mL}^{-1}$)	Amount found ($\mu\text{g mL}^{-1}$)	Recovery (%) ^a	RSD (%) ^b	Relative error
Ciprofloxacin	1.5706	1.583	100.82	2.89	0.0124
	4.7118	4.677	99.27	1.748	0.0034
	6.2824	6.310	100.44	1.276	0.0276
Pefloxacin	15.8	15.658	99.103	1.625	0.142
	31.6	31.565	99.89	1.565	0.111
	47.4	48.106	101.49	0.354	0.706
Sparfloxacin	1.86	1.822	97.96	2.31	0.038
	5.58	5.357	96.0	2.42	0.223
	7.44	7.607	102.24	1.12	0.167

^a Average of twelve determinations; ^b RSD, relative standard deviation

Table 5: Mean Recoveries of Drugs in the presence of common tablet excipients^a

Excipients	Ciprofloxacin	Pefloxacin	Sparfloxacin
Magnesium Stearate	98.2 ± 0.01	95.62 ± 0.21	102.63 ± 1.53
Lactose	102.84 ± 1.66	102.22 ± 2.13	104.93 ± 0.78
Starch	105.64 ± 1.49	97.96 ± 2.13	100.33 ± 0.78
Talc	99.69 ± 1.49	100.09 ± 2.26	97.27 ± 1.83
Gelatin	99.69 ± 1.49	95.41 ± 1.49	97.26 ± 0.77

^a n = 4

Table 6: Assay of the Quinolones in Tablets using the new method and non-aqueous titration

Drug (Amount on label ,mg)	Tablet brands	Amount found (mg)	RSD (%)	Mean % Recovery ± S. D. ^a		Statistics	
				New method	Official method	F-test	t-test
Ciprofloxacin (500 mg)	Vitapro	520.75	1.16	104.15 ± 1.21	104.54 ± 2.99	0.05	0.92
	Ciprotab	500.35	1.39	100.07 ± 1.40	102.14 ± 0.06	0.08	0.03
	Ciprocil	496.95	1.45	99.39 ± 1.45	95.03 ± 0.50	0.63	0.05
	Cenox	517.0	1.07	103.4 ± 1.11	103.71 ± 0.62	0.99	0.73
Pefloxacin (400 mg)	Peflomed	405.0	2.05	101.25 ± 2.08	102.38 ± 1.66	0.70	0.62
	Pinox	415.56	1.89	103.89 ± 1.97	104.69 ± 0.70	0.67	0.51
	Peflotab	417.04	2.73	104.26 ± 2.85	103.73 ± 0.42	0.29	0.93
Sparfloxacin (200 mg)	Sloxin-200	198.82	2.41	99.41 ± 0.24	100.45 ± 0.12	0.02	0.32
	Sparmax	200.68	0.97	100.34 ± 0.97	100.83 ± 1.33	0.32	0.78
	Sparx	202.26	0.78	101.13 ± 0.79	102.89 ± 1.90	0.08	0.62

^a Average of six determinations



The results obtained for the assessment of intra-day, inter-day accuracy and precision of the new methods are presented in Tables 2 and 3 respectively while the assessment of the method ruggedness is presented in Table 4. Accuracy of the assay procedures gave standard errors ranging from 0.012 - 0.027 (ciprofloxacin), 0.035 - 0.66 (pefloxacin) and 0.038 – 0.17 (sparfloxacin) for the intra- and inter-day assessment. The low errors observed point to the suitability of the Ce/DMAB system in assessing the quinolones.

Interference studies

Before proceeding with the analysis of the investigated drugs in their pharmaceutical dosage forms, interference liabilities were carried out to explore the effect of common excipients that might be added during formulation. Samples were prepared by mixing definite amount of the drug with various amounts of the common excipients, magnesium stearate, lactose, starch, talc and gelatin. The analyses of these laboratory prepared samples were carried out using the general procedure and the recovery values were determined. No interference was found from these excipients. The recovery values ranged from 95.41 to 105.64 % with relative standard deviations in the range 0.01 to 2.26 %. The result is presented in Table 5. This indicated the absence of interference liabilities from the excipients. Although the method based on oxidation reaction is not selective, these good recoveries ensure its suitability for the analysis of the investigated drugs in their solid dosage forms without interference from the common reducing excipients.

This proposed method also has the advantage that the measurements are performed at 470 nm in the visible region away from the UV absorbing capabilities of interfering substances that might be co-extracted from dosage forms.

Tablet analyses

The new method developed in this work for the analysis of ciprofloxacin, pefloxacin and sparfloxacin was adopted for the analysis of these drugs in tablets containing the drugs. Non-aqueous titration was utilized as the standard procedure. The results were compared statistically using F-ratio and student t-test. The results are presented in Table 6. There was found to be no significant difference in the content of these drugs when analysed by the new oxidimetric procedure and the reference non-aqueous titration using acetic perchloric acid.

Stoichiometry and Reaction mechanisms

The mole ratio method was used to study the stoichiometry between the investigated drugs and Ce (IV). The presence of many centres that are liable to oxidation in the drug molecule gave high values of the molar ratio between Ce (IV) and the drug. The molar ratio between Ce (IV) and DMAB was determined by Job's method and it was found to be 4: 1. Based on this ratio, the reaction postulated by DasGupta and Boroff³⁵

is said to proceed according to the mechanism given in Scheme 2. Treatment of DMAB with Ce (IV) yielded formic acid and *p*-dimethylaminophenol which upon further oxidation gave the corresponding quinoxaline derivative that serves as a means of measuring the excess Ce (IV) for ciprofloxacin and pefloxacin.

CONCLUSION

Ciprofloxacin and Pefloxacin were found to react readily with cerium (IV) to give a light brown colour while sparfloxacin gave a reddish brown product. The method demonstrated the usefulness of Cerium (IV)/DMAB system in the spectrophotometric analysis of ciprofloxacin and pefloxacin. The proposed method has advantages over other previously reported visible spectrophotometric method with respect to its high sensitivity, which permits the determination of very low concentration, simplicity of the procedures and reliability of the results. Furthermore, all the analytical reagents are inexpensive, have excellent shelf life and are available in any analytical laboratory. The proposed method can be applied in quality control laboratories for the routine analysis of the investigated drugs in raw materials and pharmaceutical formulation and as an in-process quality control method in pharmaceutical industries.

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