Int. J. Chem. Sci. : 1(4), 2003, 421-428

SPECTROPHOTOMETRIC DETERMINATION OF CERTAIN FLUOROQUINOLONE ANTIBIOTIC DRUGS IN PURE AND PHARMACEUTICAL FORMULATIONS

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ABSTRACT

A simple, rapid and sensitive spectrophotometric method for the determination of certain fluoroquinolone antibiotic drugs has been developed. The proposed method is based on the reaction of ciprofloxacin (CFLX), norfloxacin (NFLX), enrofloxacin (EFLX), ofloxacin (OFLX), pefloxacin (PFLX) and sparfloxacin (SFLX) with iron (III) in an acidic medium to yield yellowish orange coloured product with maximum absorption a 410, 422, 440 and 454 nm. The results of the precision and accuracy studies are presented along with optical parameters. The proposed method was applied to the determination of these drugs either in their pure form or pharmaceutical formulations. The common excipients used as additives in pharmaceuticals do not interfere with the proposed method.

Key Words: Fluoroquinolones antibiotics, Precision, Accuracy, Spectrophotometric determination

INTRODUCTION

The fluoroquinolones are antimicrobial agents used in the treatment of a variety of bacterial infections[1]. The fluoroquinolones have emerged as one of the most important classes of antibiotics of the past decade. Ciprofloxacin (CFLX), norfloxacin (NFLX), enrofloxacin (EFLX), ofloxacin (OFLX), pefloxacin (PFLX) and sparfloxacin (SFLX) are fluoroquinolone synthetic broad-spectrum antibacterial drugs. These are widely used in the treatment of respiratory tract and urinary tract infections.

Chemically, ciprofloxacin is [1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7(1- piperazinyl) quinolone-3-carboxylic acid] the reference substance for the modern fluoroquinolones that mark the beginning of a new era in antibacterial chemotherapy. It is the cyclopropyl residue at position 1 of the quinolone system that gives a considerable improvement in antibacterial activity compared with the corresponding fluoroquinolone of the ethyl residue at the same position[2]. Its mode of action is thought to be through blocking bacterial DNA replication and transcription by inhibiting DNA gyrase, ultimately giving rise to cell lysis. Because of this special mechanism of action,

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it is considered to be the most active broad-spectrum antibiotics effective against Gram-positive, - Gram-negative pathogens to combat infections caused by micro organisms that are resistant or multi resistant to other antimicrobials, such as aminoglycoside and β-lactams or tetracyclines. Enrofloxacin [1-cyclopropyl-6-fluoro-7-(4-ethyl-1-piperazinyl)-4-oxo-1,4-dihydro-3-quinolone carboxylic acid] is antibacterial agent similar to ciprofloxacin. Norfloxacin [1-ethyl -6-fluoro-1, 4-dihydro-4-oxo-7-(1-piperazinyl) quinolone-3-carboxylic acid] is an antibacterial drug active against both Gram-negative and Gram-positive micro organisms. Ofloxacin is chemically, 9-fluoro-2, 3-dihydro-3-methyl-10 (4-methyl-1-piperazinyl-7-oxo-7;H-pyrido[1,2,3,-De]-1,4-benzoxazine - 6-carboxylic acid and it is a new fluoroquinolone antibacterial agent used in the treatment of gonorrhoea. Chemically, pefloxacin is [1-ethyl-6-fluoro-7(4-methyl-1-piperazinyl)-4-oxo-1,4-dihydro-3-quinolone carboxylic acid and is in the form of its methane sulphonate, a new synthetic fluoroquinolone antibacterial agent used in the treatment of serious infections caused by resistant group of bacteria. At last, sparfloxacin is a recently developed fluoroquinolone drug, which is extremely useful in treating many infections. It is a difluorinated quinolone which was approved in 1996 for treatment of community acquired pneumonia and acute bacterial exacerbation or chronic bronchitis[3]. Chemically, it is 5-amino-1-cyclopropyl-7 (cis-3,5-dimethyl-1-piperazinyl)-6,8-difluoro-1,4-dihydro-4-oxo-3-quinolone carboxylic acid. In contrast to the other antibiotics, sparfloxacin shows a superior activity against respiratory pathogens.

Disadvantage[4] of all the fluoroquinolone antibacterial agents is that they are generally not recommended for use in children, adolescents, pregnant and breast breeding women because of their propensity to cause joint erosions in immature animals. Therapeutic drug monitoring of fluoroquinolone is most applicable when the drug in question has a narrow therapeutic range, and is used chronically has potentially toxic side effects, if overdosed. Literature reveals the determination of certain fluoroquinolones[5-9] in biological fluids. Lagana et al.[10] determined the norfloxacin in human plasma and human tissues. Mizumo et al.[11] and Miyazawa et al.[12] determined the ofloxacin in human scalp hair. These antibiotics have been determined using several analytical methods which include, colorimetry[13, 14], polarography[15, 16], HPLC[17-20], TLC[21-23] and spectrophotometry[24-27] either pure or in dosage forms. Of these, spectrophotometry is considered to be simple and economical for drug analysis. Belal et al.[28] reviewed these drugs up to 1998.

The analysis of fluoroquinolones by traditionally performed technique is slow and suffers using microbiological methods. However, these from poor precision and specificity. Although numerous chemical and physical techniques have been reported for the assay of fluoroquinolone, they suffer from a variety of disadvantages. Hence, an attempt has been made to develop a spectrophotometric method with greater precision, accuracy and sensitivity for the determination of certain fluoroquinolone antibiotics using $FeCl_3$ either in their pure form or pharmaceutical formulation and also it can be readily adopted in the forensic samples.

EXPERIMENTAL

Apparatus

A Hitachi, Model U - 2000, double beam spectrophotometer with 1cm (quartz) cells was used for measurements of absorbance.

Chemicals and Reagent

All chemicals used were of analytical reagent (AR) grade.

A 0.5% (w/v) solution of anhydrous ferric (III) chloride in 0.1N HCl was freshly prepared in distilled water.

Standard Solutions

Stock solutions of pure (1mg / mL) CFLX, NFLX, EFLX, OFLX, PFLX, and SFLX were prepared by dissolving 50 mg into 50 mL calibrated standard flask with methanol.

A working standard solution of CFLX, NFLX, EFLX, OFLX, PFLX, and SFLX containing 25-250 µg/mL were prepared by further dilution.

These solutions were stored in well-closed vessels and direct contact with light was avoided.

Determination of pure drugs

An aliquot of sample containing 1-10 mL working standard solutions of CFLX, NFLX, EFLX, OFLX, PFLX, and SFLX were transferred into a series of 25 mL standard flasks. A volume of 3 mL of 0.5% ferric chloride reagent was added to each flask. The content of the flask was shaken well and kept aside for 5-10 minutes and the volume was adjusted with 0.1N HCl. The absorbance was measured at 440 nm for CFLX, 454 nm for NFLX, PFLX and EFLX, 420 for SFLX and 410 nm for OFLX against corresponding reagent blank after 10 minutes. A calibration graph was drawn and the regression equation calculated.

Analysis of pharmaceutical formulations

Ten tablets were finely powdered and mixed. An accurately weighed quantity equivalent to 100 mg of each drug were transferred in to a 100 mL standard flask and extracted with 3 x 25 mL methanol by magnetically stirring for 15 minutes. filter and flask with methanol and completed to the mark with same solvent. It was mixed well and diluted with distilled water so that the solution contains 25-250 µg/mL of these drugs.

RESULTS AND DISCUSSION

The method is based on the formation of yellowish orange coloured chromophore with Fe (III) in acidic medium. The chromophore exhibits a maximum absorption at about 440 nm for CFLX, 422 nm for SFLX, 410 nm for OFLX and 454 nm for NFLX, EFLX and PFLX against

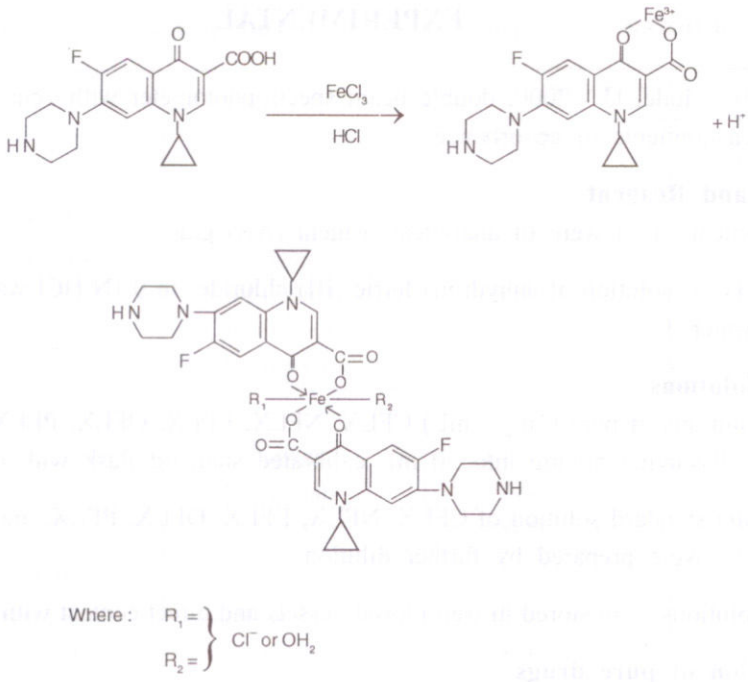

Fig. 1 Probable mechanism and structure of CFLX - Fe (III) complex.

a reagent blank. The chromophore was found to be stable for more than 24 hours. A systematic study of the effect of reagent concentration on the reaction was carried out to determine optimum concentration, which has been used in the proposed method. It was experimentally established that 3 mL of 0.5% reagent was optimal for maximum colour formation. Although lower reagent concentrations gave colours of higher intensities, then faded very quickly with time.

This mechanism is in agreement with the observation that the complex dissociates at higher acidity as a result of the reaction favouring the backward direction. It is suggested that the complexation is a fast reaction in which iron (III) directly enters the active carboxylic acid and the adjacent keto group in the 3- and 4-position, respectively, thus forming a six-membered ring according to the following scheme given in Fig.1.The complexation may occur through the carboxylic group, because as previously reported, decarboxylation is the major route of decomposition of these fluoroquinolone antibacterial drugs[29]. The probable mechanism and structure for the CFLX-Fe (III) complex is also given in Fig.1 Similar mechanism is also applicable to other fluoroquinolone NFLX, EFLX, OFLX, PFLX and SFLX. The complexation of NFLX and CFLX with Fe (III) in aqueous medium was studied using different spectroscopic techniques[30-

[33]. Other metal ions such as iron (II), copper (II), calcium (II) and aluminium (III) were investigated but all gave negative results. Iron (III) was found to react instantaneously to form a highly stable coloured complex.

In order to confirm the reliability and suitability of the proposed method, recovery studies were carried out by adding to known quantities of standard drug solution previously analysed samples and re-analysing the same by the proposed method. No interference was observed due to excipients, colour etc.; thus making it more reliable, simple and suitable for routine quantitative estimation of drug from dosage forms.

Analytical parameters, Beer's law range (μg/mL), molar absorptivity (litre $mole^{-1}$ cm^{-1}) and Sandell's sensitivity (μg/mL/cm^2) are given in Table 1 along with the slope and correlation coefficient obtained from regression equation using the least squares method. If any value of molar absorptivity is × 10^3 to 10^5, the method is considered to be a good method. One cannot go beyond 10^5 even with dyes, chromogenic ligands as instrumental accuracy for measurement of (A) is limited to 3-places of decimal for absorbance. The proposed method is about 6- 7 times more sensitive than the reported one.

Table 1. Optical characteristics, precision and accuracy data

S. No.	Drug	λ_{max}	Beer's law range (μg/mL)	Molar Absorptivity liter.$mole^{-1}$.cm^{-1}	Sandell's Sensitivity (μg/mL/cm^2)	Slope	Correlation Coefficient
1.	Ciprofloxacin (CFLX)	440	2.5-25	0.5253×10^3	0.3938	1.0015	0.9998
2.	Norfloxacin (NFLX)	454	2.5-25	0.6434×10^3	0.3099	0.9664	0.9979
3.	Ofloxacin (OFLX)	410	2.5-25	0.6707×10^3	0.3364	0.6452	0.9987
4.	Enrofloxacin (EFLX)	454	2.5-25	0.6826×10^3	0.2930	0.7984	0.9978
5.	Pefloxacin (PFLX)	454	2.5-25	0.7056×10^3	0.2950	0.8543	0.9989
6.	Sparfloxacin (SFLX)	422	2.5-25	0.7217×10^3	0.3395	0.9569	0.9991

When pharmaceutical formulations containing fluoroquinolone are analysed, the results obtained by proposed method are given in Table 2 with good agreement in labeled amounts.The present method is sensitive and simple and hence can be used in small laboratories equipped with spectrophotometers only. The proposed method was applied to various samples of these fluoroquinolone antibiotics available with market and the results obtained are summarized in Table 1. The method is precise and accurate. Hence, this approach could also be applied to detection in drug abuse cases in forensic laboratories as well as pharmaceutical formulations.

Table 2. Determination of fluoroquinolone antibiotics in pharmaceutical formulations.

S. No.	Drug*	Label Claim	Recovery** %	
			Proposed Method	Reference Method[34]
1.	Ciprofloxacin (CFLX)			
	Pure Sample	250 μg/mL	101.14	100.21
	Tablet	500 mg/tab	100.10	100.16
	Tablet	500 mg/tab	99.32	99.47
	Tablet	250 mg/tab	101.20	101.80
2.	Norfloxacin (NFLX)			
	Pure Sample	250 μg/mL	100.41	100.33
	Tablet	400 mg/tab	99.99	99.30
	Tablet	400 mg/tab	100.34	97.80
3.	Ofloxacin (OFLX)			
	Pure Sample	250 μg/mL	99.20	——
	Tablet	200 mg/tab	100.45	——
	Tablet	200 mg/tab	98.67	——
	Tablet	200 mg/tab (30 mg Dextrose + 30 mg lactose)	99.30	——
4.	Pefloxacin (PFLX)			
	Pure Sample	250 μg/mL	99.14	——
	Tablet	400 mg/tab	100.02	——
	Tablet	400 mg/tab	99.80	——
	Tablet	400 mg/tab	101.00	——
5.	Sparfloxacin (SFLX)			
	Pure Sample	250 μg/mL	100.36	100.10
	Tablet	100 mg/tab	101.20	——
	Tablet	200 mg/tab	99.30	——
	Tablet	200 mg/tab	100.55	——
6.	Enrofloxacin (EFLX)			
	Pure Sample	250 μg/mL	99.96	——

* Tablets from different manufacturers

** Each result is a mean of three replicates.

CONCLUSION

The results of the precision and accuracy studies are presented along with optical parameters. The results obtained suggest that the method is suitable for the determination of fluoroquinolones in pharmaceutical formulations without fear of interferences caused by the excipients expected to be present in such formulations. The proposed method is quite simple, sensitive, accurate, rapid, economic and reproducible. Hence, this approach could be applied to detection in drug abuse cases in forensic laboratories. Also, this procedure could be used to determine fluoroquinolones in spiked biological fluids.

ACKNOWLEDGEMENTS

The authors are very grateful to the Director, Central Forensic Science Laboratory, Bureau of Police, R&D, Govt.of India, Hyderabad (India), for providing the laboratory facilities and to Dr. Reddy's Research Foundation, Hyderabad (India) for providing pure fluoroquinolone antibiotic drugs as gift samples.

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Accepted : 6.8.2003