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Spectrophotometric Determination of Ciprofloxacin Hydrochloride in Ophthalmic Solution

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Abstract The objective of this study was to develop simple and rapid method for the determination of ciprofloxacin hydrochloride in ophthalmic solution using UV spectrophotometry. The intrinsic absorbance of ciprofloxacin was measured directly at 275 nm. The calibration graph was linear over the range 2.0–7.0 μg mL^{-1} with relative standard deviations (RSD) values ranging from 1.55 to 2.47% (n=6). The method was validated through the parameters of linearity, accuracy, precision, limit of detection, limit of quantitation,specificity and robustnessaccording to ICH. The proposed method is enabled a quantitative determination of ciprofloxacin hydrochloride in ophthalmic solution.

Keywords UV Spectrophotometry, Quinolones, Analytical Method, Ciprofloxacin Hydrochloride

1. Introduction

Ciprofloxacin (1 – cyclopropyl – 6 – fluoro-1,4 – dihydro – 4 – oxo – 7-(1-piperazinyl) – 3-quinolone carboxylic acid) (Fig. 1) is a synthetic fluoroquinolone antimicrobial agent. It is a relatively new, second-generation fluoroquinolone antibiotic with an expanded spectrum of activity against Gram-positive and Gram-negative bacteria. This antimicrobial act through the inhibition of DNA-gyrase, an enzyme that is critical to bacterial chromosome replication. Ciprofloxacin, like other fluoroquinolones, contains a piperazine group at position 7 of the 4-quinolone nucleus, which results in activity against *Pseudomonas aeruginosa*. It is used in a wide range of infections of the urinary, respiratory and gastrointestinal tracts, as well as in skin structure and ocular infections[1].

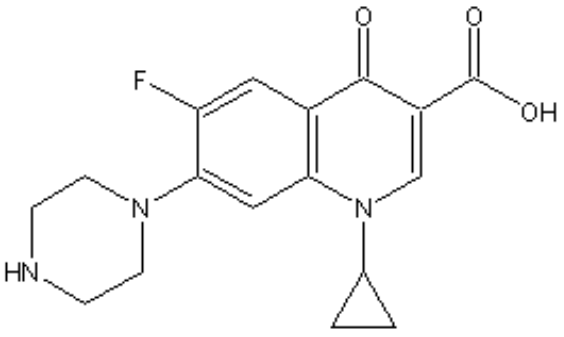

Figure 1. Chemical structure of ciprofloxacin

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It is very important to determinefluoroquinolones for the purpose of pharmaceutical quality control. Most of the analytical methods for the determination of ciprofloxacin employ HPLC[2-6], it is also the official method in United States Pharmacopoeia, British Pharmacopoeia and Brazilian Pharmacopoeia[7-9].

Numerous others methods have been reported for the determination of ciprofloxacin using techniques such as visible spectrophotometry[10-40].

Some methods for analysis of quinolones using UV spectrophotometric method were described in the literature [41-57], meantime, for determination of ciprofloxacin are relatively few ones[45, 58-62] and no method for direct quantitation of ciprofloxacin hydrochloride ophthalmic solution was found.

Spectrophotometric methods are important once they can be used in small laboratories and in places there are not sophisticated and expensive instrumentations.

The aim of this work was to develop and validate an analytical method for the determination of ciprofloxacin hydrochloride by direct measurement of its intrinsic ultraviolet absorption.The method has been successfully applied to the determination of ciprofloxacin in ophthalmic solution.

2. Material and Methodology

2.1. Reagents and Chemicals

All reagents were analytical grade and high purity water was used in all experiments. A stock standard solution of ciprofloxacin hydrochloride (100.0 μg mL^{-1}) was prepared using the standard from EMS Sigma Pharma Group (Brazil) and water. The solution was stored at 4℃. Working standard

solutions were prepared from the stock solution by appropriate dilution with water.

Ciprofloxacin hydrochloride ophthalmic solutions were obtained commercially. The ophthalmic solution were claimed to contain 3.5 mg mL^{-1} of drug and boric acid, sodium citrate, dissodiumedetate, benzalconium chloride and purity water as excipients.

2.2. Apparatus

A UV/VIS spectrophotometer KAYAK XA, HP 8453 model was used with 1 cm optical path length quartz cells for all absorbance measurements.

2.3. Standard Preparations

Aliquots of the stockstandard solution containing 100.0 µg mL^{-1} of ciprofloxacin hydrochloride were diluted to give the final concentrations of 2.0 to 7.0 µg mL^{-1}. Water was used as blank. The absorbance was measured at 275 nm.

2.4. Sample Preparations

Aliquots of ciprofloxacin hydrochloride ophthalmic solution were transferred volumetrically to give a final concentration of 3.5 µg mL^{-1}. All determinations were conducted in six replicates.

3. Results and Discussion

3.1. Spectral Characteristics

Several solvents were tested (water, methanol, hydrochloric acid 0.1 *M* and sodium hydroxide 0.1 *M*). The analytepresented maximum absorption at 275 nm in water (Fig. 2). This solvent was chosen because its excellent absorption, moreover itseconomic and low environment impacts.

3.2. Analytical Parameters

The method showed linear regression in the concentration range of 2.0 to 7.0 µg mL^{-1}(Fig.3). The precision of the proposed method was calculated by carrying out six independent assays of ciprofloxacin hydrochloride reference standard at concentration of 3.5 µg mL^{-1}. The relative standard deviation (RSD) of six assays was calculated. The inter-day variation was similarly evaluated in three different days. The RSD was 2.30% and 3.04% for intraday and inter-day, respectively. The limit of detection (LOD) and limit of quantitation (LOQ) of ciprofloxacin hydrochloride by the proposed method were determined using analytical curves according to ICH [63], using the standard deviation of y-intercept of regression equation and the slope of the calibration curves.

The proposed method was compared with others spectrophotometric methods found in the literature for the determination of ciprofloxacin (Table 1). As can be seen, the proposed method shows the highest sensitivity in terms of the apparent molar absorptivity (ε), so allowing analysis of lower concentration of ciprofloxacin.

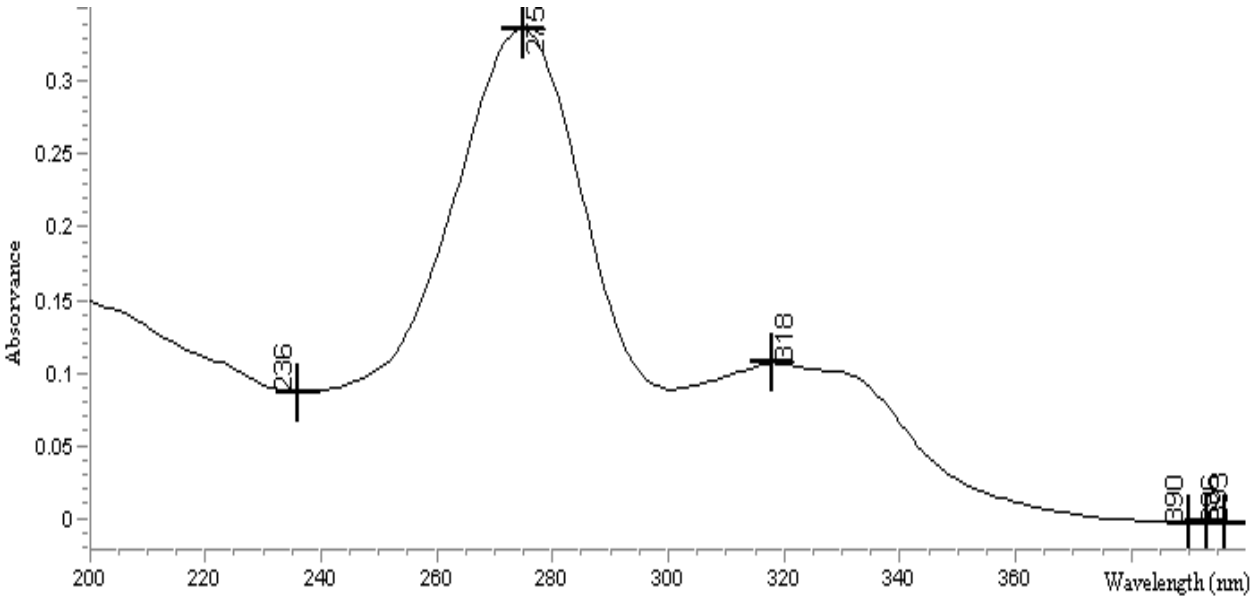

Figure 2. Spectra absorption of ciprofloxacin hydrochloride in aqueous solution

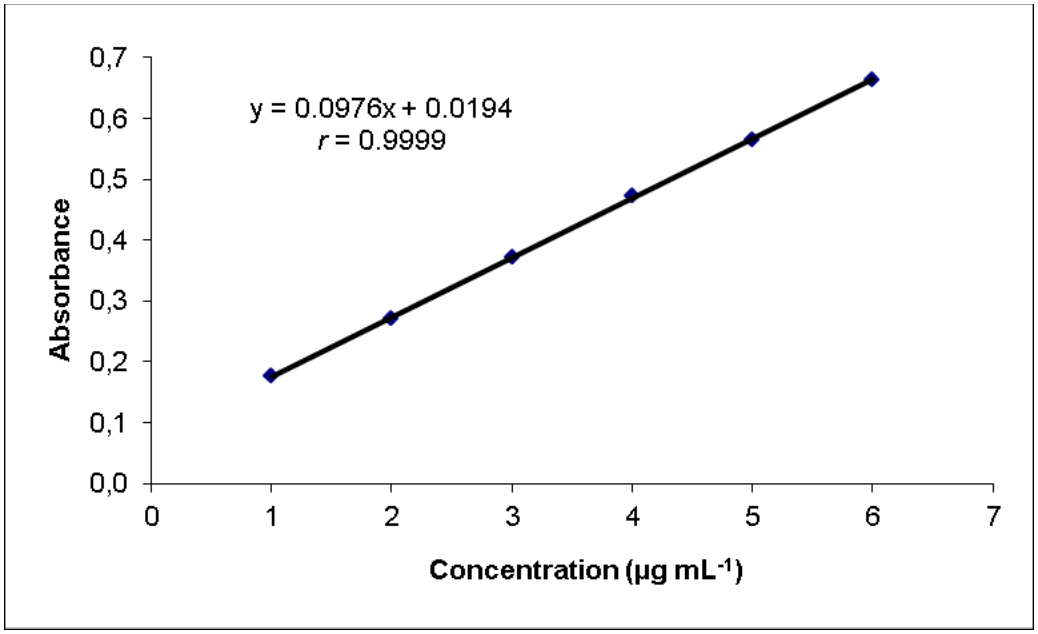

Figure 3. Standard curve of ciprofloxacin hydrochloride

Table 1. Comparison of spectrophotometric methods for ciprofloxacin determination

Reagent	λ_{max} (nm)	ε (L mol^{-1} cm^{-1})	Linear range (µg mL^{-1})	Remarks	Reference
Iron (III) – MBTH*	425	-	6.0-12.0	Involves contact time, less stable color, expensive reagent	[10]
p-benzoquinone	495	-	-	Involves rigid pH control	[11]
Iron (III) chloride	440	1.84 x 10^3	40-100	Involves heating/cooling and use of DMSO	[12]
Iron (III) in sulfuric acid media (FIA –Flow Injection Analysis)	447	-	50-500	Uses flow injection automated assembly, less sensitive	[14]
Bromocresol Purple	410	1.7 x 10^4	1.5-16.5	Involves rigid pH control and liquid–liquid extraction	[15]
Bromophenol Blue	410	1.6 x 10^4	1.5-12.0	Involves rigid pH control and liquid–liquid extraction	[15]
Iron (III) nitrate	375	-	35-300	Less sensitive	[16]
Supracene violet 3B	575	8.62 x 10^3	2.5-30	Involves liquid–liquid extraction	[17]
Eosin and palladium	545	3.4 x 10^4	3.0-10.0	EDTA inference in analysis	[18]
Iron (III) nitrate	435	-	20-100	Reaction time of 15 min	[19]
Iron (III) in sulfuric acid media (SIA - Sequential Injection Analysis)	447	-	50-500	Users system sensitive	[20]
TCBQ*	376	1.27 x 10^4	0.9-25	Involves heating	[21]
Cerium (IV) sulphate	345	5.1 x 10^3	12-120	Involves extraction into $CHCl_3$	[22]
DDQ*	460	5.00 x 10^3	5-50	Involves contact time	[23]
TCNQ*	843	1.88 x 10^4	1.5-15	Involves contact time	[23]
CL*	550	1.25 x 10^3	20-200	Involves contact time	[23]
p-nitrophenol	403	3.9 x 10^4	0.1-18	Involves heating/cooling	[24]
Sudan III	566	2.38 x 10^4	0.4-10.4	Involves heating/cooling samples	[26]
MBTH* and cerium ammonium sulphate	630	-	10-50	Involves contact time and use of solvents	[27]
Cu (II)	284	1.9 x 10^5	0.035-0.120	Derivative spectrophotometry	[28]
Chloranilic acid	520	1.39 x 10^4	16-96	Involves contact time	[29]
Tetracyanoethylene	335	2.9 x 10^5	0.25-15	Involves contact time	[29]

Table 1. Comparation of spectrophotometric methods for ciprofloxacin determination (continue)

Reagent	λ_{max} (nm)	ε (L mol^{-1} cm^{-1})	Linear range (µg mL^{-1})	Remarks	Reference
Fe(III)-1,10-phenanthroline	510	3.4 x 10^4	0.04-7.2	Involves contact time	[30]
Fe(III)-bipyridyl	522	2.95 x 10^4	0.05-9	Involves contact time	[30]
Brilliant blue G	610	2.86 x 10^4	500-600	Involves liquid-liquid extraction	[31]
Eosin Y	547	3.56 x 10^4	2-8	Involve use of solvents	[32]
Menbromin	545	1.23 x 10^4	2-15	Involve use of solvents	[32]
Bromocresol green in dichloromethane	412	2.28 x 10^4	1-20	Involves liquid-liquid extraction	[33]
FIA UV spectrophotometry	277	-	0.5-10	Expensive equipment	[60]
Solid-phase UV	277	1.3 x 10^6	0.05-0.30	Involves extraction in solid-phase	[61]
Co (II) tetrathiocyanate	623	8.38 x 10^2	20-240	Involves rigid pH control and liquid-liquid extraction	[34]
Bi (III) tetraiodide	453	-	5-80	Involves precipitation and filtration	[35]
Ammoniumvandate	766	-	10-40	Involves heating/cooling samples	[36]
Ce (IV) sulphate–MO*	520	6.1 x 10^4	0.5-3.5	Involves contact time	[37]
Ce (IV) sulphate–IC*	610	3.0 x 10^4	1.0-7.0	Involves contact time	[37]
NBD-Cl*	477	2.66 x 10^4	3.0-18.0	Involves contact time and heating	[38]
Sudan II	550	5.3 x 10^4	0.5-8.0	Involves contact time	[39]
Congo red	517	2.83 x10^4	0.5-6.0	Involves contact time	[39]
Gentian violet	585	2.21 x10^4	0.5-10	Involves contact time	[39]
UV spectrophotometric	275	9.0 x 10^4	2.0-7.0	Non-stringent working conditions, no heating or no extraction step, no involves contact time, highly sensitive	Present method

*MBTH, 3-methyl-2-benzothioazolin-2-one-hydrazone; TCBQ, Tetrachlorobenzoquinone; DDQ, 2,3-dichloro-5,6-dicyano-p-benzoquinone; TCNQ, 7,7,8,8-tetracyanoquinodimethane; CL, p-chloranil; MO, Methyl orange; IC, Indigo carmine; NBD-Cl, 4-chloro-7-nitrobenzo-2-oxa-1,3-diazole.

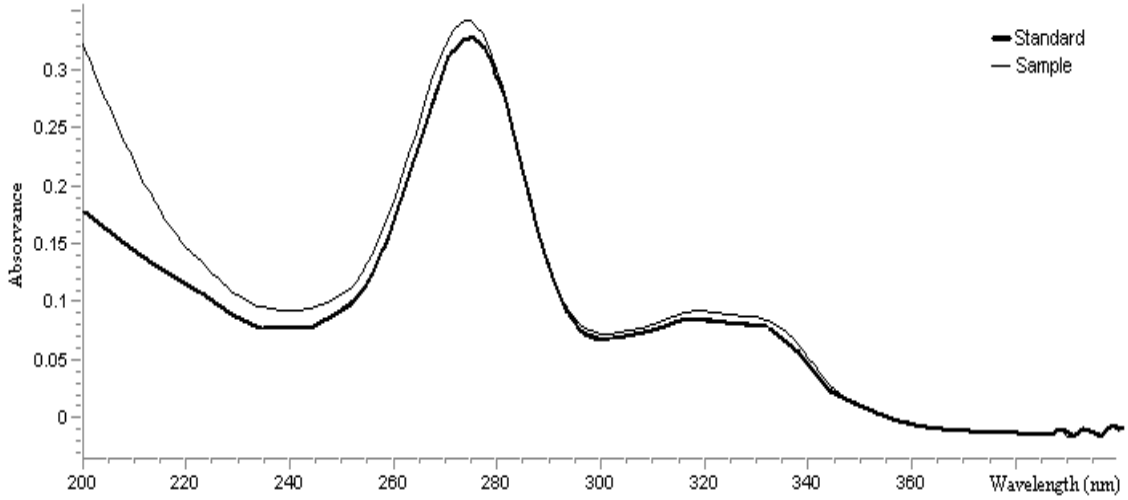

Figure 4. Superposition of standard and ophthalmic solution spectrograms of ciprofloxacin hydrochloride

The proposed method was applied to the analysis of ophthalmic solution commercially available containing ciprofloxacin hydrochloride. Satisfactory results were obtained (Table 2).

The accuracy of the method was also confirmed by performing recovery studies. This was carried out by adding known amounts of ciprofloxacin hydrochloride standard solution to the samples at three levels. All solutions were

prepared in triplicate and analyzed. The percentage recovery was calculated and the results are presented in Table 3.

Table 2. Results of ciprofloxacin hydrochloride ophthalmic solutions quantitation

Day	Amount of ciprofloxacin in formulation[a]		Mean amount ± S.E.M.[b]	RSD (%)
	µg	%		
1	3.55	101.34		
2	3.48	99.35	99.79 ± 0.0016	2.44
3	3.45	98.67		

[a]Mean value of three determinations.
[b] Standard Error Mean

Table 3. Results of recovery studies

	Amount of pure ciprofloxacin added (µg mL^{-1})	Total found (µg/mL)	Recovery (%)*	Mean Recovery (%)
R1	1.25	1.28	102.31	
R2	2.25	2.28	101.45	101.51
R3	3.25	3.28	100.77	

*Mean value of three

The results presented suggest that there is no interference from any excipient which are present in the pharmaceutical commercial samples (Fig. 4). Furthermore, the specificity was proved by the *t* and F-tests.The *t* and F values calculated, 1.95 and 1.32, respectively, are below tabulated values. The tabulated *t* and F values at 95% confidence limit are *t*=2.18 and F=4.28.

4. Conclusions

The proposed UV spectrophotometric method shows interesting features such as rapidity, simplicity, high sensitivity and low cost per analysis, involving neither sophisticated nor expensive instrumentation. The procedure is, therefore, suitable for the determination of ciprofloxacin hydrochloride in ophthalmic solution as a routine method with adequate accuracy and precision.

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