DIRECT AND DERIVATIVE SPECTROPHOTOMETRIC DETERMINATION OF GEMIFLOXACIN MESYLATE IN PURE FORM AND PHARMACEUTICAL PREPARATIONS USING π ACCEPTORS.

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ABSTRACT

Two simple, quick and sensitive methods were described for the spectrophotometric determination of Gemifloxacin mesylate (GFX) either in pure form or in the Pharmaceutical form. The methods were based on the reaction of GFX as 'n' electron donor with Chloranilic acid(CLA) and Parachloranil(CL) as π acceptors to give highly coloured complex species. The coloured products were quantitated spectrophotometrically at 530nm and 540nm at zero order, 590and 610nm for the first derivative and 630and 650nm for second order derivative. Optimization of the different experimental conditions were described. Beer's law was obeyed in the concentrations range of 10to60µg/ml, 5to25µg/ml at zero order,5to 25µg/ml, 5to40µg/ml at first order and 2to20µg/ml and 2to 14µg/ml at second order. The limit of detection and quantification were calculated and relative standard deviation (RSD) for different concentrations of GFX using various acceptors were less than 0.128 %.The association constant of 1:1 complexes and Standard free energy changes were studied. The proposed methods were successfully applied to the determination of GFX in pharmaceutical dosage form without interference from common additives encountered.

Key words : Gemifloxacin mesylate, charge transfer complex, spectrophotometer, pharmaceutical formulation

INTRODUCTION TO GEMIFLOXACIN

Gemifloxacin (GMF) chemically R,S-7-(3 amino methyl 4- syn methoxyimino-1pyrrolidinyl)-1cyclopropyl-6-flouro1,4,dihydro 4- oxo-1,8 napthyridine-3-carboxylic acid methane- sulphonate[1-3] is a new flouroquinolone antibacterial compound with enhanced affinity for bacterial topoisomerase-IV and is being used for the treatment of respiratory and urinary tract infections.[4-6]. Literature review revealed few analytical methods for the determination of GMF include HPLC, electrophoresis, UV-spectrophotometry concerning visible spectrophotometry. very few methods have been reported and no derivative spectrophotometry has been reported, hence sensitive and accurate ,direct, derivative spectrophotometric method has been viewed[7-10].The molecular interaction between electron donor and electron acceptor generally associated with formation of intensely coloured charged complexes(Ion paired complexes) had absorbed radiation in visible region [11]. The photometric methods

based on these interactions were usually simple and convenient because of the rapid formation of complexes .GFX is a good 'n' electron donor and form ion pair complex with π acceptors such as Chloranilic acid(CLA) and Para chloranil(CL) and known to yield ion pair complexes and radical anions with variety of electron donor[12-22]. This donor acceptor interactions has been investigated, GFX as electron donor. The present study describes direct simple, more sensitive and precise spectrophotometric methods than the existing U.V and HPLC methods that were free from experimental variables such as extraction steps. No interference was observed in the assay of GFX from common excipients in levels used in the pharmaceutical formulations. The reaction conditions and application of the method for the determination of GFX in Tablets have been established. In addition the association constant, the stiochiometric ratio of reactants and standard free energy changes were determined.

EXPERIMENTAL

APPARATUS: All absorption spectra were made using SCIMADZU U.V 160spectrophotometer equipped with 10mm matched Quartz cells.

MATERIALS AND REAGENTS: All reagents and solvents were of Analytical grade(AR grade)

The following commercial formulations were subjected to the Analytical procedures

1.Gemez(Majesta) a division of Glenmark Pharmaceuticals Ltd, Mumbai, labeled to contain 320mg of GFX

2. G.cin(Hetero drugs Ltd, Hyderabad, labeled to contain 320mg of GFX were used.

STOCK SOLUTIONS: Stock solutions of GFX were prepared by dissolving 40mg and 10 mg in 5ml of distilled water and volume was made up to mark in a100ml calibrated volumetric flask with distilled water to obtain the stock solution of $1x10^{-3}$M and 0.1mg/ml of drug. Such drug solutions were stable for about 3days when stored in refrigerator at 5^{o}c
Chloranilic acid (CLA) and P-Chloranil(CL) were freshly prepared as $1x10^{-2}$ M solution in acetonitrile .The solution was stable for greater than one week at 5^{o}c

GENERAL PROCEDURES: To a 10ml volumetric flask transferred 1ml of $1x10^{-3}$M GFX stock solution and 2ml of $1x10^{-2}$M CLA or CL reagents were added .The reaction mixture was heated on a water bath at 60 ± 2^{o} C for 10min for CLA or CL methods. After cooling and diluting to volume with acetonitrile, the absorbance was measured at 530nm and 540nm for CLA and CL respectively against a reagent blank prepared in the same manner.

PROCEDURE FOR DOSAGE FORM: An accurately weighed amount of finely powdered tablet equivalent to 100mg of drug was dissolved in about 20ml of distilled water and transfered into a 100ml calibrated volumetric flask and after 30 min of mechanical stirring was filtered into a 100ml calibrated volumetric flask through Whatmann No:41 filter paper necessary amounts of filtrate was diluted to 100ml with acetonitrile and then the same procedure is followed as described above.

STIOCHIMETRIC RELATIONSHIP: Job's method for continuous variation[23] was employed to establish the stiochiometry of coloured products. A $1x10^{-3}$M concentration standard solution of GFX and $1x10^{-3}$M concentration solution of CLA and CL were used .The absorbance of the complex was used to calculate the association constants and standard free energy changes of complexation(ΔG).

RESULTS AND DISCUSSIONS :

The interaction of GFX with CLA and CL in acetonitrile yielded red coloured chromgen absorbing maximally at wavelength 530nm and 540nm respectively (Fig:2) Most propably due to the formation of charge transfer complexes between GFX acting as n-donor(D) or Lewis base and CLA and CL act as π acceptor(A) or Lewis acid.

$D^{\cdot\cdot} + A \leftrightarrow (D^{\cdot\cdot} \rightarrow A)$ <polar solvent> $D^{\cdot+}A^{\cdot-}$

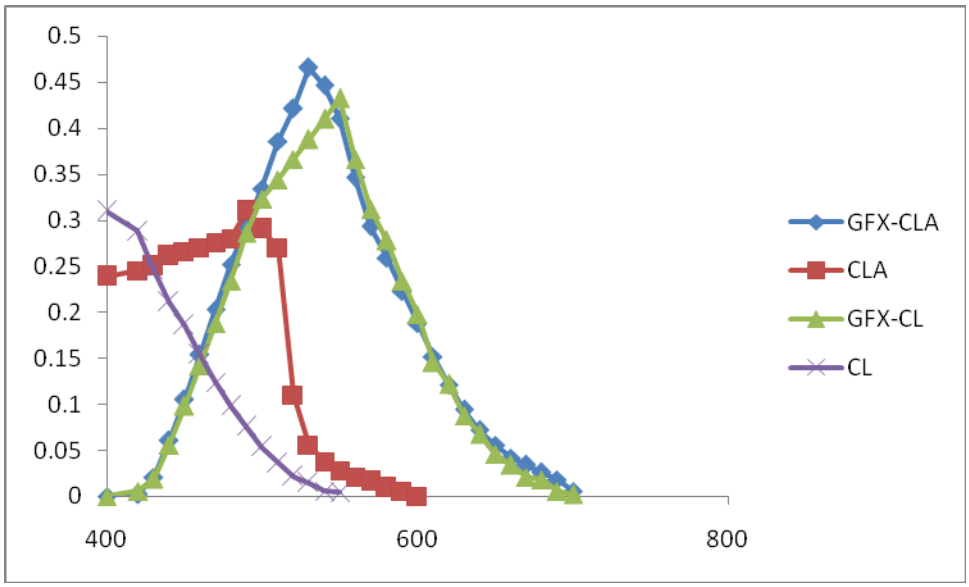

Fig-1 Absorption spectra of 40µg/ml GFX complex with $1x10^{-2}$-M CLA and CL respectively against reagent blank.

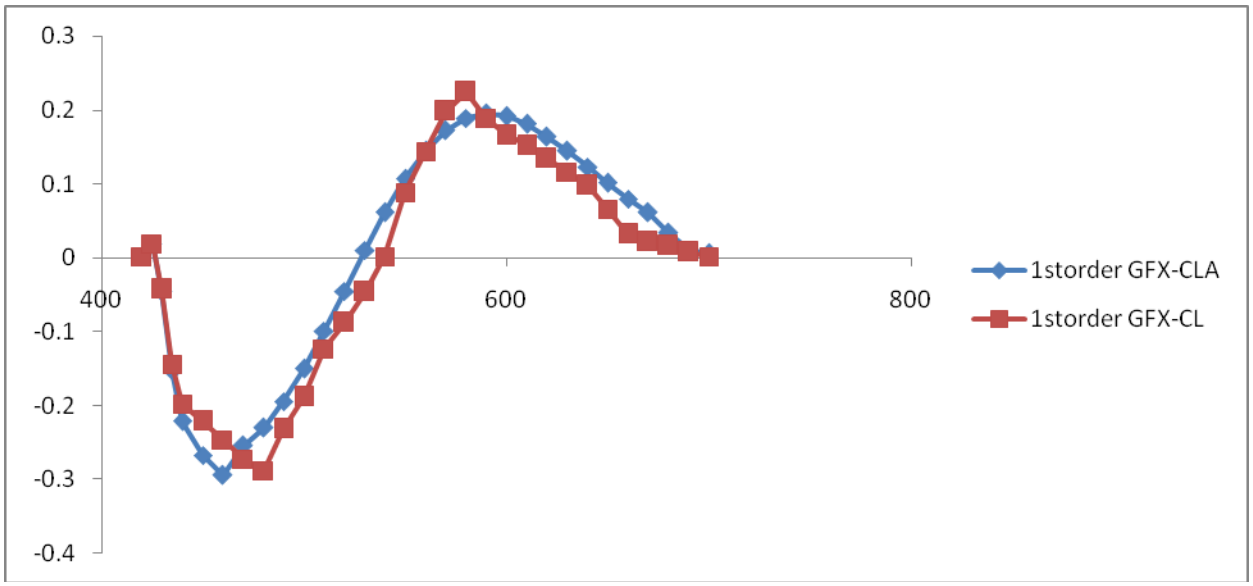

Fig2: First order derivative of 40µg/ml GFX complex with $1x10^{-2}$-M CLA and CL

The dissociation of the DA complex was promoted by the high ionizing power dielectric constant of acetonitrile solvent,[24], further support for the assignment was provided by the comparison of the absorption bands with those of the $CLA^{\cdot-}$ and $CL^{\cdot-}$ radical anions produced in acetonitrile

OPTIMUM CONDITIONS

1) CHOICE OF THE SOLVENT: Although charge transfer complexes are propably formed in many solvents the high cut of points of some solvents observed short wavelengths and therefore clear cut spectroscopic evidences for charge transfer complexes could not be ascertained. Acetonitrile is found to be the best solvent for the two reagent because it has high relative permittivity which yielded maximum of $CLA^{\cdot}$ and

CL· species . Other solvents such as Ethanol, Chloroform, Methylene chloride and 1,4 –Dioxane were also examined as possible substitutes but the colour intensity was lower with these solvents.(Table-1)

Table-1 Effect of solvent on the absorption intensity of the reaction products of GFX with CLA and CL

Solvent	Absorbance CLA(530nm)	Absorbance CL(540nm)
Acetonitrile	0.814	0.515
Chloroform	0.016	0.01
Ethanol	0.026	0.023
1,4Dioxane	0.022	0.002

2) REAGENT CONCENTRATION : When the various concentrations of CLA and CL were added to a fixed concentration of GFX, 2ml of $1x10^{-2}$M solution of CLA or CL found to be sufficient for the production of maximum and reproducible colour intensity (Fig-3). Higher concentrations of reagents did not effect the colour intensity.

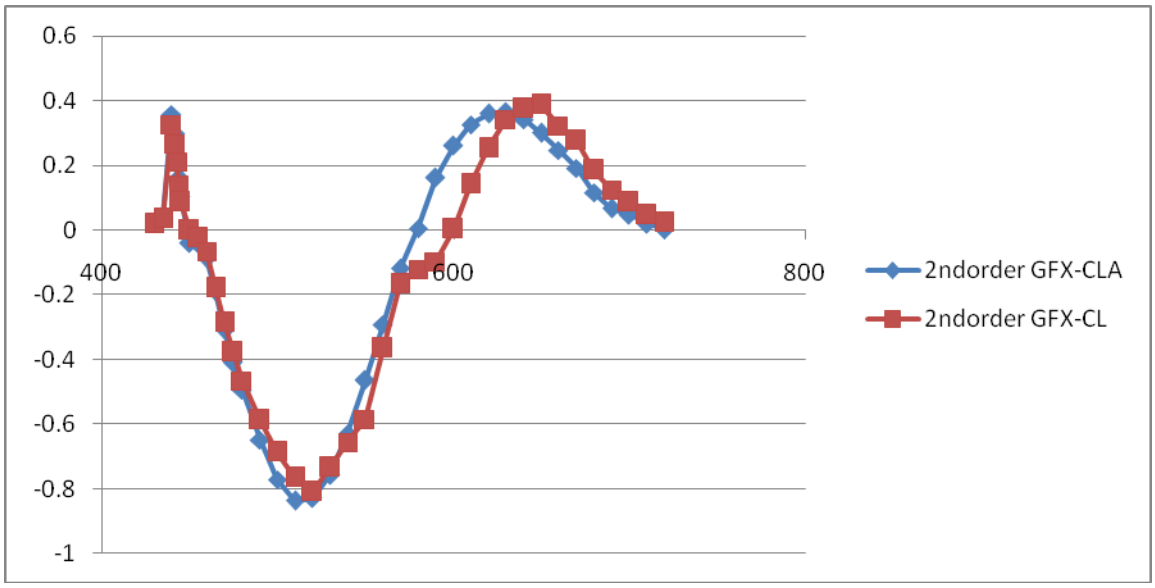

Fig-3 Second order derivative of 40µg/ml GFX complex with $1x10^{-2}$-M CLA and CL

3) REACTION TIME: The optimum reaction time was determined by the following colour development at room temperature (25±2 ^{0}C). Complete colour development was not attained till 60 minutes, after heating on waterbath at 60±2^{0}C for 10 minutes for CLA and CL. The colour remain stable for ≥ 36 hours for the two reagents.

4) STICHIOMETRIC OF THE REAGENTS : The stichiometric ratio of the reagents (drug: reagent) in the charge transfer complex was determined by the method of continuous variation(Job's method) was found to be 1:1 for CLA and CL (Fig-4).This suggests that one Nitrogen atom of the piperidine ring in GFX is involved in the reaction with CLA and CL.

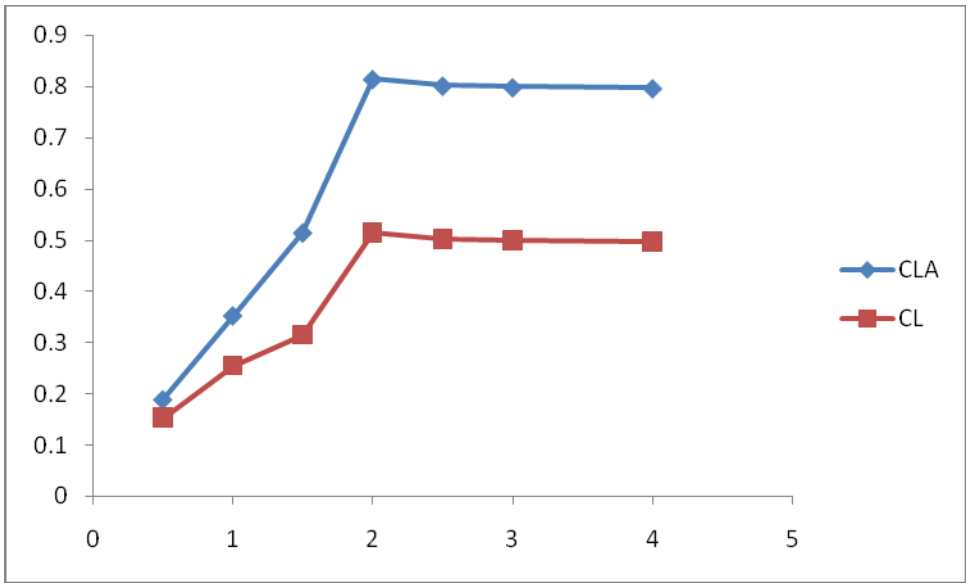

Fig-4 Effect of reagent concentration($1x10^{-2}$M)on the formation of GFX complex with CLA and CL

Table-2Statastical Analysis of Calibration graphs and analytical data for the complexation of GFX with CLA and CL methods

Parameter	**GFX-CLA**	**GFX-CLA Ist derivative**	**GFX-CLA 2^{nd} derivative**	**GMF-CL**	**GMF-CL Ist derivative**	**GMF-CL 2^{nd} derivative**
Wavelength ƛ - max	530nm	590nm	630nm	540nm	610nm	650nm
Temperature	60^0C	60^0C	60^0C	60^0C	60^0C	60^0C
Time	10min	10min	10min	10min	10min	10min
Linearity range(µg/ml)	5-25	5-40	2-20	10-60	5-50	2-14
Molar absorptivity €	$1.165x10^5$	$4.875x10^4$	9.12510^4	$1.025x10^5$	$5.6x10^4$	$9.7x10^4$
LOD(mcg/ml)	1.2	0.9	0.7	1.5	1.1	0.9
LOQ(mcg/ml)	3.95	2.73	2.35	5.2	3.8	2.73
Slope	0.011	0.004	0.008	0.011	0.006	0.009
Intercept	0.001	0.001	0.002	0.01	0.002	0.001
Correlation coefficient	0.997	0.999	0.998	0.996	0.999	0.999
Accuracy	100.03	100.15	100.3	100.01	100.05	100.1
RSD%	0.324	0.096	0.106	0.0089	0.0107	0.1289

5) ASSOCIATION CONSTANT AND FREE ENERGY: By using job's method it was found that 1:1 complex was found and K the Association constant was calculated based on the formula:

$K=1-\alpha/\alpha^2$ X C where $\alpha = O.D_{max}-O.D_{min}/O.D_{max}$

And C is the Concentration.

The calculated association constants were recorded in Table-3 .The low values obtained are common in these complexes due to the dissociation of the original donor-acceptor complex to the radical anion.

Table 3: Stability constants of Gatifloxacin mesylate chelate with CLA and CL byJob's method

Parameters	GMF-CLA at 530nm	GMF-CL at 540nm
Total molar conc	$1x10^{-4}$M	$1x10^{-4}$M
N	2.303	2.303
A/A_{ex}	1.0714	1.11
β*	$2.156x10^{5}$	$9x10^{6}$
Logβ	5.34	6.954
ΔG^0	-3.9	-3.99

The standard free energy changes of complexation (ΔG^0) were calculated from the association constants by the following equation[25]

$$\Delta G^0 = 2.303RT \text{ Log } K$$

where ΔG^0 is the free energy change of the complex (KJ mol^{-1}),R is the gas constant(1.987cal $mol^{-1}deg^{-1}$) ,T is the temperature in Kelvin($273+000^0$C) K is the Association constant of the drug-acceptor complex ($1mol^{-1}$)

6) INTERERENCE STUDY: Potential interference by the excipients in the dosage form was also studied samples were prepared by mixing fixed amounts of common excipients such as lactose, microcrystalline cellulose, talc, magnesium sterate and starch. The good percentage recoveries (Table-4) were obtained indicating no interference was observed from any of these excipients with the prepared method. The absence of interference from these excipients was attributed to the extraction with organic solvents prior to analysis.

Table 4: Determination of Gemifloxacin in presence of common excipients

	Recovery±%RSD	
Excipient	**GFX-CLA 530nm**	**GFX-CLA 540nm**
Microcrystallinecellulose	100.05±0.1257	100.01±0.1343
Lactose(10mg)	100.03±0.159	99.8±0.1
Talc(10mg)	100.01±0.177	99.9±0.124
Magnesium sterate(10mg)	99.9±0.137	99.95±0.15
Starch(10mg)	100.01±0.157	100.03±0.1699

7) QUANTIICATION: Commercial formulations of GFX were successfully analysed by the proposed methods. The reliability of the proposed method was checked by standard addition method. The results (Table-5) show that the mean recoveries were found in the range of 100.01-100.03 with RSD≤ 0.0687 % for CLA and 100.01 with RSD≤ 0.00578 for CL. The performance order of the proposed methods is CLA > CL.

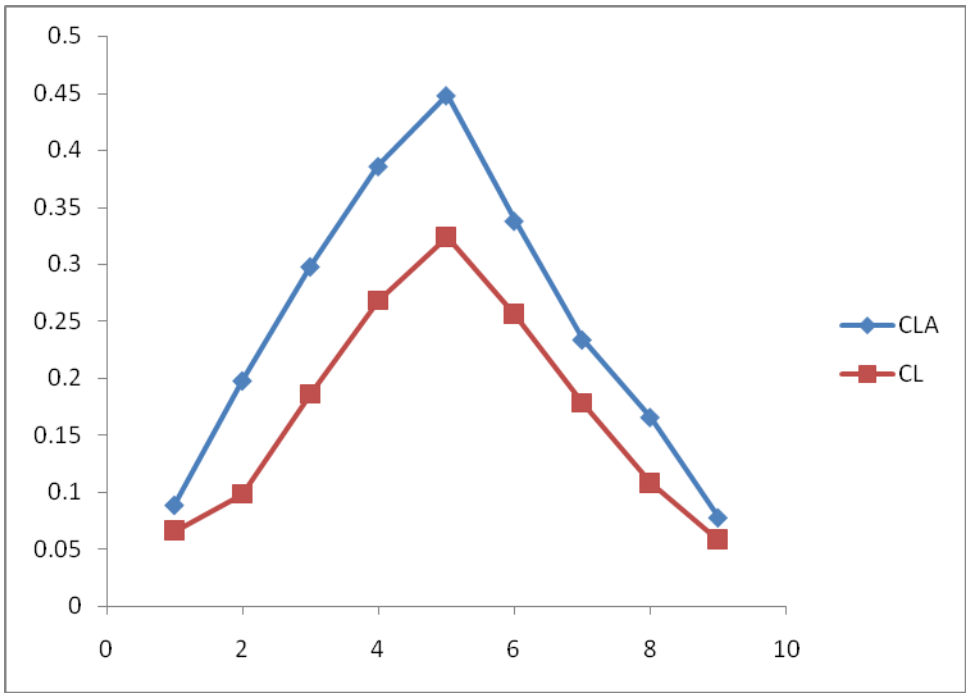

Fig-5 Job's method for GFX complex with CLA and CL;**a**-530nm,540nm,respectively.

Table-5 Results of analysis of Tablet formulations containing GFX

Formulation	Labelled amount	% Recovery CLA	%Recovery CL	%RSD CLA	%RSD CL
Gemez	320mg	100.03	100.01	0.0687	0.003726
G-cin	320mg	100.01	100.01	0.01117	0.005773

Table-6 Determination of GFX in Pharmaceutical dosage forms applying standard addition technique

Proposed method	Taken µg/ml	Added µg/ml	Gemez	G-cin
CLA	40		100.03	100.01
		40	99.8	99.6
		80	100.02	99.8
		120	100.02	100.03
		160	100.03	100.04
		200	100.02	100.02
Mean			99.98	99.91
%RSD			0.08361	0.1639
CL				
	40	--	100.02	100.01
		40	99.8	100.03
		80	100.02	99.9
		120	100.04	99.8
		160	100.01	100.03
		200	100.03	100.02
Mean			99.98	99.97
%RSD			0.0839	0.0865

CONCLUSION : The proposed methods are simple ,less time consuming and more sensitive and validated than the reported U.V and HPLC methods. The proposed method concerning CLA is superior to CL. The proposed methods are suitable for the determination of GFX in pharmaceutical formulation without interference from excipients suggesting useful applications in bulk analysis.

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