ISSN 2321-807X
Volume 12 Number21
Journal of Advances in Chemistry

Validated Spectrofluorimetric Determination of Gemifloxacin in Pharmaceutical Preparations and Spiked Human Plasma.

Reem N. El Gammal, Mohammed E.A. Hammouda*, Dalia R. El-Wasseef and Saadia M. El-Ashry.
Department of Medicinal Chemistry, Faculty of Pharmacy, University of Mansoura, 35516, Mansoura, Egypt.
Corresponding author: Mohammed El-Sayed Abd El-Ghaffar Hammouda
Email address: drmo4u@yahoo.com

Abstract:

A simple, sensitive and rapid spectrofluorimetricImethod was developed and validated for the determination of Gemifloxacin mesylate (GFX), in bulk powder, pharmaceutical preparations and biological fluids. The proposed method was based on complexation of GFX with $AlCl_3$ as complexing agent then measuring the fluorescence of the resulted complex after enhancement with sodium dodecyl sulphate (SDS) in borate buffer (pH 8) at emission wavelength of 401 nm after excitation at 264 nm. Different experimental parameters affecting relative fluorescence intensity (RFI) were carefully studied and optimized to obtain the maximum relative fluorescence intensity. The developed method was validated according to International Conference on Harmonisation guidelines in terms of specificity, linearity, lower limit of quantification (LOQ) 0.54 $ng.ml^{-1}$, lower limit of detection (LOD) 0.18 $ng.ml^{-1}$, accuracy and precision. The proposed method was found to be rectilinear over the concentration range of 1-20 $ng.ml^{-1}$ with recovery percentage of 99.85±0.84. The proposed method was applied successfully for the determination of GFX in pharmaceutical preparations and spiked human plasma with recovery percentage of 99.97±0.79 and 99.96±1.73 respectively. The results were statistically analyzed and compared with a reference method and no significance difference was found between both methods.

Keywords: Gemifloxacin mesylate, Spectrofluorimetry, $AlCl_3$, SDS, Complexation, Pharmaceutical preparations and Biological Flulds.

Introduction:

GFX is chemically structured ((*R, S*)-7-[(4*Z*)-3-(aminomethyl)-4-(methoxyimino)-1-pyrrolidinyl]-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acid methanesulfonate **Fig. 1**. It is a fourth generation synthetic fluorinated quinolone antibacterial agent. It is present in two forms, either as free GFX base or as GFX mesylate salt [1]. GFX has a broad spectrum antibacterial activity against gram-positive and gram-negative organisms due to its dual mechanism of action by inhibition of both DNA gyrase and topoisomerase IV enzymes. The 4-oxo-3 carboxylic acid group is essential for the antibacterial activity, because they mediate binding to the DNA-gyrase complex [2].
Several methods were declared for determination of GFX in pharmaceutical preparations or human plasma including: spectrophotometry [3-8], spectrofluorimetry [9-12] capillary electrophoresis [13, 14], HPIC methods [8, 15-20] and HPTlC methods [16, 21-23].
Among available methods for determination of drugs, spectrofluorimetry is considered the most popular technique for determination of pharmaceutical compounds due to its simplicity, specificity and low cost.

Objective of the work:

The purpose of this method is to establish a simple, sensitive and rapid spectrofluorimetric method for determination of GFX by complexation with Al^{+3} and enhancement of fluorescence intensity of the formed complex by addition of SDS. The proposed method was validated according to lCH guidelines and also applied for determination of GFX in tablets and spiked plasma.

Experimental

Appapratus:

- Spectrofluorimetric analysis were carried out on a RF-1501 Shimadzu spectrofluorimeter with a Xenon arc lamp, and a 1cm quartz cell.
- A Consort P-901 pH-meter was used for pH measurements.

Reagents and Materials:

All the chemicals used were of analytical reagent grade, and the solvents were of spectroscopic grade.

- GFX was kindly supplied by Medizin Pharmaceutical Company (Borg Elarab, Alexandria, Egypt) and its pharmaceutical preparation Quinabiotic Tablets, with Batch No: 12625 were obtained from local pharmacy.
- Aqueous solution of $AlCl_3$ (1×10^{-3}M), SDS 0.5% with 99% purity were supplied from Park Scientific limited, Northampton, UK. Borate buffer (0.2 M), acetate buffer (0.2 M).
- Human plasma was provided by Mansoura Univ**e**rsity Hospitals (Mansoura, Egypt) and kept frozen until used after gentle thawing.

Sample preparation and procedures:

Stock solution of GFX mesylate (100 µg.ml^{-1}) was prepared by dissolving 10 mg GFX in 100 ml measuring flask and the volume was completed to 100 ml with distilled water. Serial dilutions were performed to cover the working range.

Procedures:

Construction of calibration curve:

Into a series of 10 ml volumetric flasks, transfer aliquots of the working solution (100 ng.ml^{-1}) so as to obtain drug concentration in the range of 1-20 ng.ml^{-1}. Add 1.2 ml (1×10^{-3} M) $AlCl_3$, 2 ml of borate buffer (pH 8) then add 0.5 ml of SDS (0.5%), shake well and leave the solution for 20 min at room temprature. Complete to volume with borate buffer and measure the resulting flourescence intensity at emission wavelength 401 nm after excitation at 264 nm, plot the concentration versus RFI to obtain the standard calibration curve.

Procedure for tablets dosage form:

Five tablets are ground to a fine powder, transfer a weighed amount **o**f the powdered tablets equivalent to 10 mg of the drug into a 100 ml volumetric flask, then mix with 50 ml distilled water and sonicate the flask for 30 min. Dilute the solution to the mark with distilled water, mix well and filter. Prepare serial dilutions covering the working concentration range of 1-20 ng.ml^{-1}. Standard addition method was applied as follow:

Adding a known amount of pure drug at three different concentrations 1, 4, 6 ng.ml^{-1} to a previously analyzed tablet solution at three different concentrations 1, 2, 3 ng.ml^{-1}. The concentrations mentioned of the pure drug added in separate flasks to each of the mentioned tablet concentration and the solution was reanalyzed for the total drug content. The fluorescence intensity is then measured for all solutions and the data is plotted against concentration of the standard added, linear regression is performed and the slope and intercept of the calibration curve are used to calculate the unknown concentration of analyte in th**e** provided sample.

Assay of GFX in spiked human plasma:

Transfer 1 ml of plasma sample into centrifugation tube then add 1 ml of the studied compound containing 100 µg.ml^{-1}. Shake well for 3 min, then extract with 3×5 ml of acetonitrile for protein denaturation. Shake the mixture on a vortex mixer for 30 second, and centrifuge for 12 min at 4000 rpm in a microcentrifuge. Transfer the protein free supernatant into 25 ml volumetric flask and dilute to the mark with distilled water and then proceed as described under "construction of calibration curve".

Results and discussion:

GFX exhibits strong intense native fluorescence in Acetonitrile at" 401 nm after excitation at 264 nm. Complexation of GFX with Al^{+3} results in increase in RFI upon adding SDS (0.5%) and borate buffer pH 8 there is enhancement of RFI by 100% **Fig. 2**, The complex is formed through binding of Al^{+3} to the carbonyl and carboxylate oxygen of the drug which is essential for activity, forming six-membered ring as shown in **Fig. 3**. On the other hand, the piprazinyl substituent has a natural chair conformation which is not favorable for fluorescence. Protonation of piperzinyl nitrogen stabilizes planar configuration, therefore both binding effect of Al^{+3} and protonation of piprazinyl imino group lead to a stable planar structure and extended π-electron resonance, which enhances fluorescence properties of the drug. [24]

Different experimental parameters affecting RFI of GFX were carefully studied and optimized, each factor was changed individually which others were kept constant as follow:

Effect of pH: The effect of pH on RFI was studied using acetate and borate buffers. It was found that higher RFI was obtained in alkaline pH. By using different pH values of 0.2 M borate buffer it was observed that; pH 8 gave the maximum RFI, and at higher pH values there was a sharp decrease in RFI as shown in **Fig. 4**. So pH 8 was the optimum pH for measurement throughout the whole work.

Effect of $AlCl_3$ Volumes: By changing the volume of $AlCl_3$ (1×10^{-3}M) it was found that 1.2 ml gave the maximum RFI as shown in **Fig. 5**.

Effect of SDS Concentration: RFI of the reaction product of GFX and Al^{+3} was enhanced by addition of SDS. By using different SDS concentration, it was observed that 0.5 ml of 0.5% SDS was the optimum for obtaining maximum RFI as shown in **Fig. 6**, also in absence of SDS there was a sharp decrease in RFI of the resulting complex.

Effect of reaction time: The maximum RFI was obtained after 15 minutes and remained constant for 60 minutes, so 20 minute was selected as optimum reaction time and used throughout the whole method.

Stoichiometry of the reaction :

The stoichiometry of the reaction was studied by using limiting logarithmic method [25]. The two straight lines obtained using increasing concentrations of the reagent while keeping the concentration of the drug constant and using increasing concentration of drug while keeping the concentration of the reagent constant. Plots of log [RFI] versus log [GFX] and log [$AlCl_3$] gave two straight lines, the value of their slope were 0.2 for GFX and 0.21 for $AlCl_3$ **Fig. 7a, 7b**. Hence, it is concluded that the reaction proceeds in ratio of 1:1 confirming that one molecule of GFX reacts with one molecule of the Al^{+3}.

Validation of the proposed method:

The proposed method was validated through the validation criteria including: linearity, sensitivity (detection limit & quantitation limit), specificity, accuracy, intraday and ineterday precision, and robustness.

Linearity: Linear relationship was obtained for GFX by plotting RFI against drug concentrations. Linear relationship was obtained over the concentration range cited in (Table 1). Linear regression analysis of the data by the proposed methods gave the following equation:

$$RFI = 88.77 + 31.64C \quad (r = 0.9999)$$

Where RFI is the relative fluorescence intensity, C is drug concentration (ng/ml) and r is correlation coefficient.

The data were analyzed statistically [26] giving acceptable high value of the correlation coefficient (r) of the regression equation, small values of the standard deviation of residuals ($S_{y/x}$), intercepts (S_a), and slopes (S_b), and small values of the percentage relative standard deviation and percentage relative errors.

The Sensitivity of method was evaluated by determination of limit of detection (LOD) and limit of quantitation (LOQ) according to ICH guidelines [27].

LOD was evaluated by determination minimum amount of the analyte in the sample which can be detected but not necessarily quantitated as an exact value; on the other hand IOQ was evaluated by determination of the minimum amount of the analyte in the sample which can be quantitatively determined with suitable precision and accuracy. Both LOQ and LOD were calculated using the following equations and the results were presented in (Table 1):

$$LOQ = 10\ S_a/b \text{ and } LOD = 3.3\ S_a/b$$

Where S_a = standard deviation of the intercept and b = slope of the calibration curve.

Accuracy and precision: The results of the assay of GFX were compared with those obtained by the comparison method [12] which is based on measurement of native fluorescence of GFX in isopropanol at 400 nm after excitation at 272 nm. Statistical analysis of the results using student"s *t*-test and variance ratio *F*-test proved that there was no significant difference between the performance of the proposed and comparison methods regarding the accuracy and precision, respectively (Table 2). The intraday and interday precision for the reaction was obtained by analysis of the samples at 3 different concentrations in one day and for three successive days, and the results were cited in (Table3).

Robustness of the method is evaluated by the regularity of the RFI with minor changes in experimental variables. Such as time of reaction (20±2 min) volume of $AlCl_3$ (1.2±0.2 ml). These minor changes of parameters didn't affect the RFI.

Assay of the dosage forms:

Standard addition method was applied to establish the concentration of the analyte that is in a complex matrix, due to presence of other components that interfere with the analyte signal causing inaccuracy in the determined concentration (Table 4).

Assay of spiked plasma:

The proposed method was applied for the determination of GFX in biological fluids, the results are summarized in (Table 5).

Conclusions:

Simple, sensitive and rapid spectroflourimetric method was established for determination of GFX in tablet and spiked plasma through complexation with $AlCl_3$ as complexing agent. The proposed method was validated according to ICH guidelines.

References:

1. Al-Hadiya BMH and Mahmoud AMM, Gemifloxacin: Analytical profiles of drug substances, excipients, and related methodology.ed. Harry GB Academic Press 2011; 36: 151-168.
2. Domagala JM, Structure-activity and structure-side-effect relationships for the quinolone Antibacterials. J Antimicrob Chemother 1994; 33: 685-706.
3. Ambadas, Rote R and Pingle SP, Validated UV-spectrophotometric methods for determination of gemifloxacin mesylate in pharmaceutical tablet dosage forms. E J Chem, 7, 2010, 344-348.
4. Abdel Wahed MG, El Sheikh R, Gouda AA, and Abou Taleb S, Kinetic spectrophotometric determination of gemifloxacin mesylate and moxifloxacin hydrochloride in pharmaceutical preparations using 4-Chloro-7-nitrobenzo-2- oxa-1, 3-diazole. J Spectro, 2014, 2014, 1-12.
5. Hajera K and Zaheer Z, Development and Validation of a dissolution test with spectrophotometric analysis for

gemifloxacin mesylate and ambroxol hydrochloride in tablet dosage form. J Pharm Tech Res, 4, 2012, 661-668.
6. Madhuri D, Kottapalli CB, Devanna N and Somasekhar G. Direct and derivative spectrophotometric estimation of Gemifloxacin Mesylate by Chelation with Cr(III) Ion. Rasayan J.Chem, 3, 2010, 9-15.
7. Madhuri D, Kottapalli CB, Devanna N and Somasekhar G, Direct and derivative spectrophotometric estimation of Gemifloxacin by chelation with Palladium (II) Ion. Rasayan J.Chem, 3, 2010, 159-165.
8. El-Bagary RI, Abo-talib NF and N. Eldin MB, Validated Stability Indicating Assay of gemifloxacin by different chromatographic and spectrophotometric methods of analysis. J. Chem Pharm Res, 3, 2011, 562-570.
9. Atia NN, Mahmoud AM, El-Shabouri SR and El-Koussi WM, Two validated spectrofluorometric methods for determination of gemifloxacin mesylate in tablets and human plasma. Int J Anal Chem, 2013, 2013, 1-11.
10. Kepekci TSE and Önal A, Spectrofluorimetric methods for the determination of gemifloxacin in tablets and spiked plasma samples. J Fluoresc., 21, 2011, 1001-7.
11. Moussa BA, Mahrouse MA, Hassan MA and Fawzy MG, Spectrofluorimetric determination of gemifloxacin mesylate and linezolid in pharmaceutical formulations. Acta Pharm, 64, 2014, 15-28.
12. Al-Tamimi SA, Alarfaj NA, Aly FA and Al-Mohaimeed AM, Spectrofluorimetric analysis of gemifloxacin mesylate in pharmaceutical formulations. Lumin, 29, 2014, 127-31.
13. Elbashir AA, Professor Saad B, Ali ASM, Al-Azzam KMM and Aboul-Enein HY, Validated stability indicating assay of gemifloxacin and lomefloxacin in tablet formulations by capillary electrophoresis. J Liq Chromatogr Rel Techno, 31, 2008, 1465-1477.
14. Paim CS, Führ F, Todeschini V, Steppe M and Schapoval EES, Simultaneous analysis of gemifloxacin mesylate and its main synthetic impurity by an optimized capillary zone electrophoretic method. Anal. Methods, 6, 2014, 1657- 1665.
15. Mohammad Y, Kumar BP, Hussain A and Harish, Development and validation of RP-HPLC method for the estimation of gemifloxacin mesylate in bulk and pharmaceutical dosage Forms. E J Chem, 7, 2010, 1621-1627.
16. Rote AR and Pingle SP, Reverse phase-HPLC and HPTLC methods for determination of gemifloxacin mesylate in human plasma. J Chromatogr, 877, 2009, 3719-3723.
17. Al-Hadiya BM, Khady AA and Mostafa GA, Validated liquid chromatographic-fluorescence method for the quantitation of gemifloxacin in human plasma. Talanta, 83, 2010, 110-6.
18. Ranjane PN, Gandhi SV, Kadukar SS and Bothara KG, Stability indicating RP-LC method for the determination of gemifloxacin mesylate. Chromatographia, 71, 2010, 1113-1117.
19. Panda SS, Ravi Kumar BVV, Mohanta G and Patel PK, Reverse phase ultra fast liquid chromatographic method for determination of gemifloxacin mesylate in tablet dosage. J PharmaSciTech, 2, 2012, 20-25.
20. Roy B, Das A, Bhaumik U, Sarkar AK, Bose A, Mukharjee J, Chakrabarty US, Das AK and Pal TK, Determination of gemifloxacin in different tissues of rat after oral dosing of gemifloxacin mesylate by LC-MS/MS and its application in drug tissue distribution study. J Pharm Biomed Anal, 52, 2010, 216-26.
21. Mahmoud AM, Atia NN, El-Shabouri SR and El-Koussi WM, Development and validation of stability indicating HPTLC assay for determination of gemifloxacin mesylate in dosage forms. Am J Anal Chem, 6, 2015, 85- 97.
22. El-Koussi WM, Atia NN, Mahmoud AM and El-Shabouri SR, HPTLC method for direct determination of gemifloxacin mesylate in human plasma. J Chromatogr B, 967, 2014, 98-101.
23. Raja T and Rao AI, Development and validation of HPTLC method for the simultaneous estimation of gemifloxacin mesylate and ambroxol hydrochloride in bulk and tablet dosage form. Anal Chem Letters, 2, 2012, 152-158.
24. Rizk M, Belal F, Ibrahim F, Ahmed S and El-Enany N, Spectrofluorimetric analysis of certain 4-quinolone in pharmaceutical and biological fluids. Pharm. Acta Helv, 74, 2000, 371-377.
25. Rose, J, "Advanced Physicochemical Experiments", Pitman,london, (1964) p. 67.
26. Miller JC and Miller JN, Statistics and chemometrics for analytical chemistry, 5th ed. Harlow, UK: Pearson Education, (2005) 39–73,107–49.

International Conference on Harmonization: Harmonized Tripartite Guideline, Validation of Analytical Procedures: Text and Methodology, Q2(R1), Current Step 4 Version, 2005 [Available from: http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q2_R1/Step4/Q2_R1__Guideline.pdf] , date accessed on 25-9-2016.

Tables:

Table 1. Analytical performance data for the spectrofluorimetric determination of GFX by the proposed method:

Parameter	Results
Wavelenght λex/λem nm	264/401
Linearity range (ng/ml)	1-20
Intercept(a)	88.77
Slope(b)	31.64
Correlation coefficient(r)	0.9999
SD of residuals ($S_{y/x}$)	2.54
SD of intercept (Sa)	1.72

SD of slope (Sb)	0.15
%RSD	0.84
%Error	0.29
LOD (ng/ml)	0.18
LOQ (ng/ml)	0.54

Table (2): Assay results for the spectrofluorimetric determination of GFX in pure form by the proposed and comparison methods

Pure form	**% Found of Drugs**	
	Proposed method	**Comparison method**
	98.72	100.97
	98.96	99.03
	100.75	100.19
	100.85	
	99.64	
	100.30	
	99.15	
	100.40	
Mean (x̄) ± S.D.	99.85 ± 0.84	100.06 ± 0.98
***t*-test**	0.37 (2.26)	
F-value	1.36 (19.35)	

Table (3): Accuracy and precision data for the determination of GFX in Pure form by the proposed method:

Parameter		**GFX concentration (ng.ml^{-1})**		
		8	**10**	**14**
Intraday	**Mean (x̄)**	100.58	98.06	102.41
	S.D.	0.46	0.32	0.69
	% RSD	0.45	0.32	0.67
	% Error	0.26	0.19	0.39
Interday	**Mean (x̄)**	100.98	97.85	102.03
	S.D.	0.60	0.66	0.47
	% RSD	0.59	0.67	0.46
	% Error	0.35	0.39	0.27

Table (4): Assay results for the determination of GFX in its tablet dosage form by the proposed and comparison methods:

Dosage form	**% Found**	
	Proposed method	**Comparison method [12]**
	99.20	100.89
	100.77	99.11
	99.93	100.318
Mean (x̄) ± S.D.	99.97 ± 0.79	100.06± 0.89

***t*-test**	0.14 (2.78)
F-value	1.30 (19.00)

Table (5): Assay results for the determination of GFX in spiked human plasma using the proposed spectrofluorometric method

	Amount taken (ng/ml)	Amount found (ng/ml)	% Found
	8	**7.88**	**98.46**
	10	**10.19**	**101.86**
	14	**13.94**	**99.57**
Mean ($\bar{x}$)			**99.96**
± SD			**± 1.73**
% RSD			**1.74**
% Error			**1.001**

Figures:

Fig. (1): Structural formula of GFX.

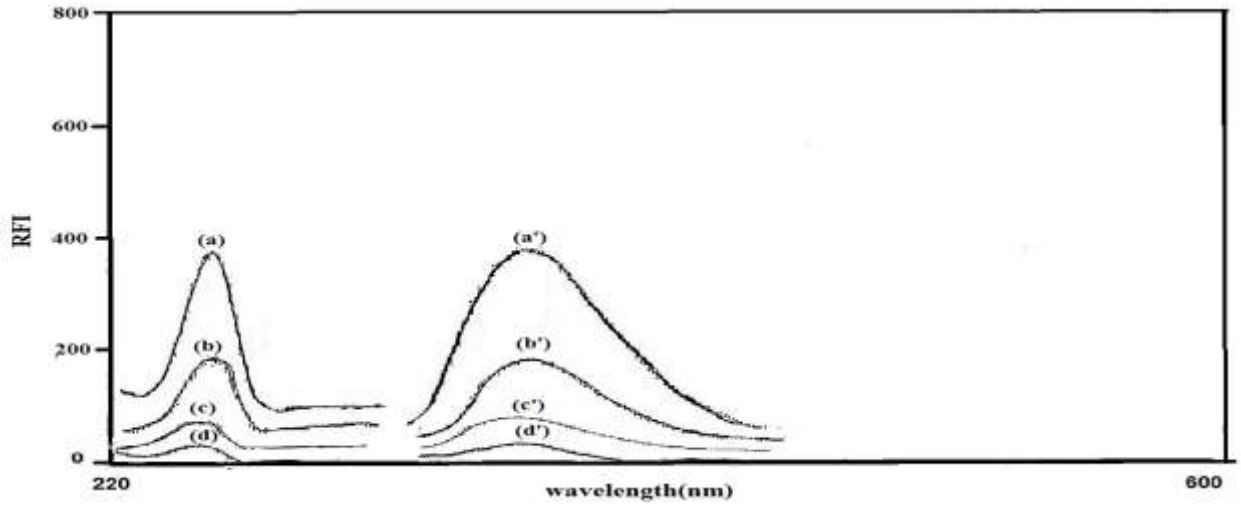

Fig. (2): (a, a') excitation and emission spectra of GFX-Al^{+3}-SDS complex (10 $ng.ml^{-1}$) while (b, b') excitation and emission spectra of GFX in acetonitrile, (c, c') excitation and emission spectra of GFX-Al^{+3} complex and (d,d') excitation and emission spectra of blank(SDS-Al^{+3}).

ISSN 2321-807X
Volume 12 Number21
Journal of Advances in Chemistry

Fig. (3): Formation of Drug-Al^{+3} complex.

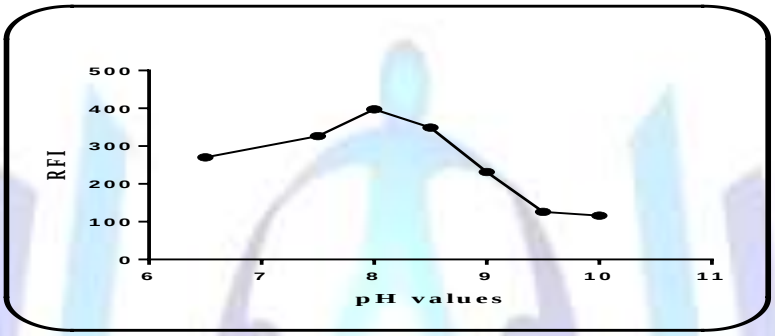

Fig. (4): Effect of pH on the fluorescent product of GFX (10 $ng.ml^{-1}$) Al^{+3}-SDS.

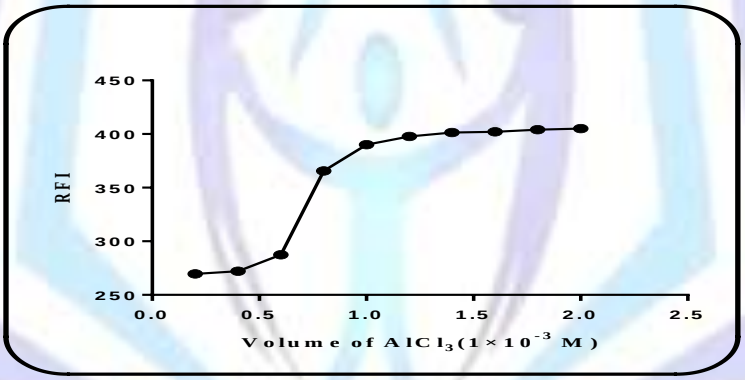

Fig. (5): Effect of different volume of $AlCl_3$ (1×10^{-3} M) on fluorescent intenisty of the reaction with GFX (10 $ng.ml^{-1}$) upon using 0.5 ml 0.5% SDS and Borate buffer (pH 8).

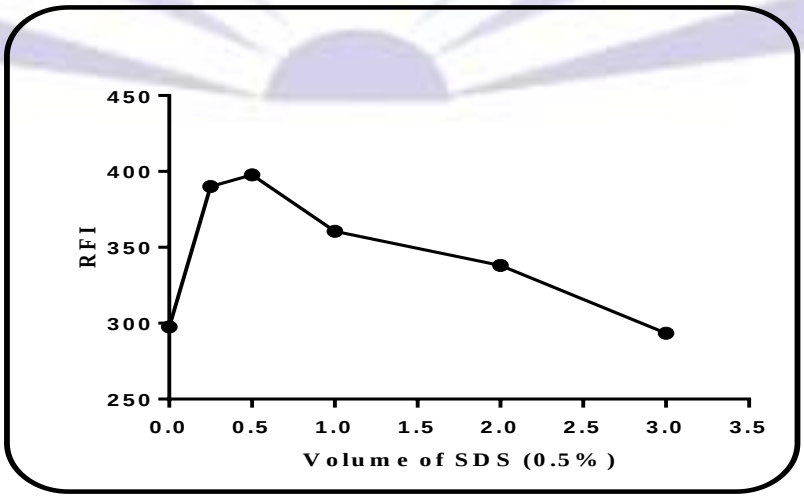

Fig. (6): Effect of SDS volume (0.5%) on fluorescence intensity of the reaction with GFX (10 $ng.ml^{-1}$) upon using 1.2 ml $AlCl_3$ (1×10^{-3}) and borate buffer (pH 8).

ISSN 2321-807X
Volume 12 Number21
Journal of Advances in Chemistry

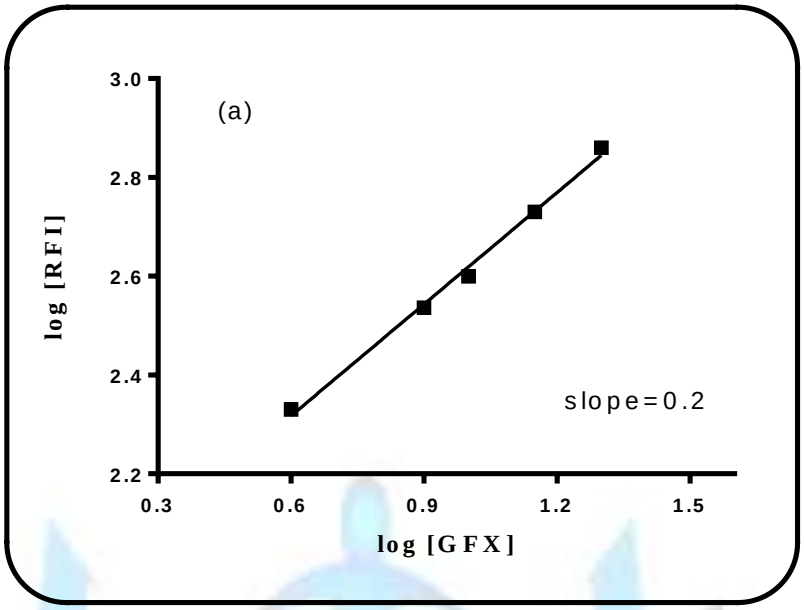

Fig. 7a

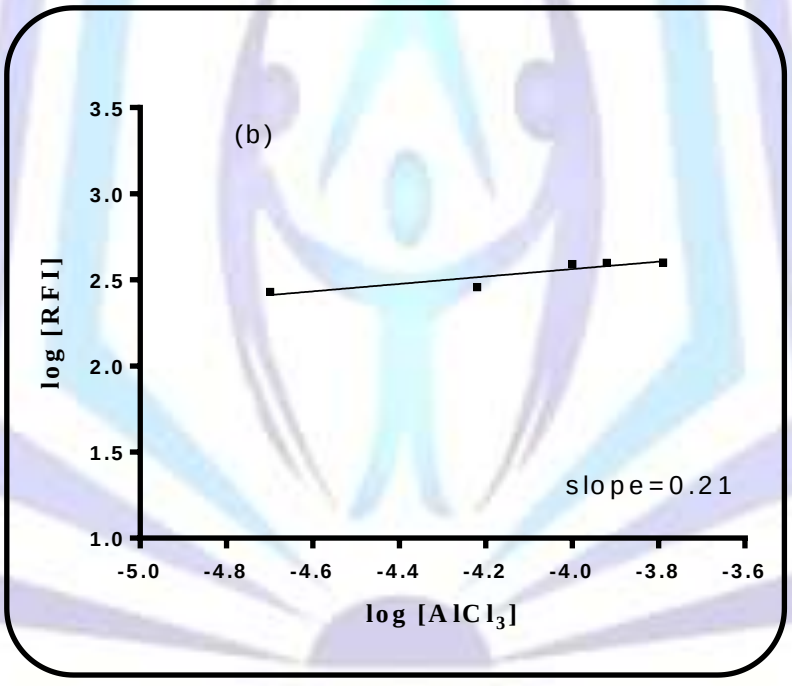

Fig. 7b

Fig. (7): Limiting logarithmic plots for molar ratio. (a) log RFI vs. log [GFX] with log [$AlCl_3$] kept constant. (b) log RFI vs. log [$AlCl_3$] with log [GFX] kept constant.