wjpmr, 2021,7(4), 219-227

SJIF Impact Factor: 5.922

WORLD JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.wjpmr.com

Research Article

ISSN 2455-3301

WJPMR

DEVELOPMENT OF DIFFERENT SPECTROPHOTOMETRIC TECHNIQUES FOR GEMIFLOXACIN MESYLATE AND CEFDITOREN PIVOXIL QUANTIFICATION IN BULK AND COMMERCIAL FORMULATIONS

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Article Received on 27/01/2021 **Article Revised on 17/02/2021** **Article Accepted on 07/03/2021**

ABSTRACT

Background: The current study aims to suggest three different accurate spectroscopic approaches to quantify gemifloxacin mesylate (GFX) using aluminum chloride (Method A and B) and cefditoren pivoxil using cerium (IV) sulfate (Method C). **Methods:** The methods are conducted by reacting aluminum chloride with gemifloxacin mesylate in alkaline medium of borate buffer pH 8 and cefditoren pivoxil (CFP) using cerium (IV) sulfate the absorbance was recorded at 270 and 509 nm, respectively. **Results:** Linear relationships were observed by using 1-10 and 2-24 µg mL^{-1} for GFX and 2-14 µg mL^{-1} solutions for CFP. The estimated quantification limits were 0.281, 0.577 and 0.533 µg mL^{-1} whereas, limits of detection values were 0.093, 0.191 and 0.176 µg mL^{-1} for the three suggested approaches, respectively. All experimental conditions, including the optical properties were studied. **Conclusion:** The suggested methods can be exploited in the routine analysis of the studied analyts in their pure and commercial products and also for quality control of both drugs.

KEYWORDS: Cefditoren pivoxil, Gemifloxacin mesylate, Spectrophotometry, Commercial formulations.

INTRODUCTION

Today antimicrobials or antibiotics are compounds often recommended in the treatment of bacterial infection or infectious diseases.[1] Antibiotics have been classified into two groups according to their activity spectrum, narrow or broad.[2] Newer compounds have been developed such as broad spectrum β-lactam cephalosporin, quinolones and fluoroquinolones antibiotics.[3]

Gemifloxacin mesylate (GFX) is a synthetic oral administration broad-spectrum fluorinated quinolones antibacterial agent used to inhibit DNA synthesis, prevent cellular replication and bacterial growth.[4] It is well known that Streptococcus pneumonia has been showed double mutations in both DNA gyrase and topoisomerase IV and they are resistant to most fluoroquinolones.[5] Thus, GFX is known as the only fluoroquinolone that posses the ability to inhibit both enzyme systems at therapeutic relevant drug levels in S. pneumonia.[6] Further, it has a minimum inhibitory concentration values that are still in the suitable range for some of these double mutants (Figure 1a).[7,8]

Cefditoren pivoxil (CFP) is an oral administration, 3rd generation and semi-synthetic cephalosporin antibiotic. It has a high potential against microorganisms. It is recommended for in the treatment of mild-to-moderate pneumonia, chronic bronchitis, acute pharyngitis/tonsillitis, acute sinusitis, and skin infectious diseases (Figure 1b).[9]

The reviewing survey addressed several analytical techniques for the determination of gemifloxacin mesylate and cefditoren pivoxil including spectroscopic methods,[10-18] electrochemical methods,[19-22] chromatographic separation.[23,26]

Although the chromatographic and electrochemical techniques have been provided certain sensitivity and accuracy for the determination of the selected drugs, they still associated with some drawbacks such as they require long analytical time, consume large amounts of solvents and need high technical skills. Therefore, the development of more simple, accurate and less time consuming spectroscopic technique is still in concern.

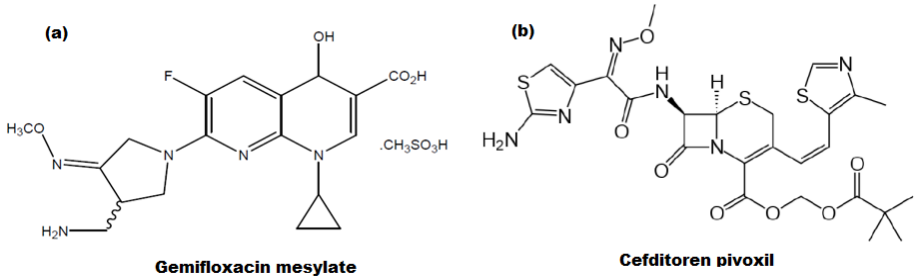


Figure 1: Structural formulas of (a) gemifloxacin mesylate and (b) cefditoren Pivoxil.

The formation of a complex becomes generally the association of two or more interacting molecules or ions. Thus, a complex is a species with well-defined substrate-to-ligand stoichiometry that can be formed in an equilibrium process.[27] Metal ions and ligand anions or molecule interaction often produces a change in color that is suitable for quantitative estimation of pharmaceutical analysis.[28]

The aim of this current study is to suggest new and easy detectable analytical probes for the quantification of GFX and CFP in Bulk and their commercial formulations. The investigation includes the usefulness of direct and derivative spectrophotometric methods for quantification of selected antibiotics. Also, it is concerned with evaluation of all experimental parameters that may affect the accuracy, precision and selectivity of the suggested analytical methods. The validation of the developed methods was conducted in accordance with the approved guidelines.

Experimental

Instrumentation: Spectrophotometer of model UItrospec 2000, (Biochrom Ltd. Cambridge, UK) was used for all spectrophotometric measurements.

Chemicals and reagents: Pure gemifloxacin mesylate, cefditoren pivoxil were gifted by Saudi Arabia Co. of pharmaceutical industries (Tabuk). Their commercial products (Factive®320 mg/tablet) and (Meiact®200 mg/tablet) were purchased from local drug stores. Aluminum (III) chloride ($AlCl_3$), acetic acid, sodium hydroxide, hydrochloric acid, disodium hydrogen phosphate, Cerium (IV) sulfate, sulfuric acid and tris hydroxymethylaminomethane were purchased from (BDH, Pool, UK). Sodium acetate, methyl orange, boric acid, phosphoric acid and borax were acquired from (Winlab, East Midland, UK).

Analytical solutions: Two standard solutions of (GFX in distilled water) and (CFP in 1.0 mol L^{-1} sulfuric acid) having 100 µg mL^{-1} were prepared. The prepared solutions were kept in the refrigerator for two weeks (GFX) and one week (CFP), respectively. Working standard solutions for each drug were obtained by making further dilution with distilled water.

General procedures: Three different procedures were proposed for the verification of the tested drugs (Methods A and B) for GFX and method C for CFP. In 10-mL measuring flasks, the tested solutions of GFX (1-10 µg mL^{-1}) and (2-24 µg mL^{-1}) were mixed with 2 mL of (1.0×10^{-2} mol L^{-1}) $AlCl_3$ and 2 mL borate buffer (pH 8) for methods A and B, respectively, then 10 mL was completed with distilled water. The formed product absorbance was deliberated at 270 nm to determine A and ΔA for the above mentioned methods, respectively. The calibration curves were plotted and the corresponding regression equations were also, calculated.

Method C: Accurately measured aliquots of CFP (2-14 µg mL^{-1}) were mixed with 1.0 mL of 5 mol L^{-1} sulfuric acid and 1.0 mL of 500 µg mL^{-1} cerium (IV) sulfate solution. The mixture was shaken and kept at room temperature for 40 min. After adding 1.0 mL of 200 µg mL^{-1} MO, the prepared solution was diluted with the same solvent. The absorbance of each solution was deliberated after 5 min at 509 nm vs. a blank prepared simultaneously.

Analysis of GFX and CFP in tablets: Not less than ten tablets of each drug (Factive®320 mg/tablet) and (Meiact®200 mg/tablet) were accurately weighed and milled well. An equivalent quantity of 10.0 mg of GFX or CFP was dissolved separately in 100-mL measuring flasks using distilled water or acidic water (10:50 v/v water: sulfuric acid), respectively. After sonication for 30 min, the contents of the flasks were filtered and measured as previously mentioned in general procedures for the three proposed spectrophotometric methods.

RESULTS AND DISCUSSION

GFX demonstrates a limited absorption peak at 270 nm. This study revealed that GFX can form an instantaneously stable complex with $AlCl_3$, in the alkaline borate buffer medium. The complex exhibited maximum absorbance peak at 270 nm (Figure 2a).

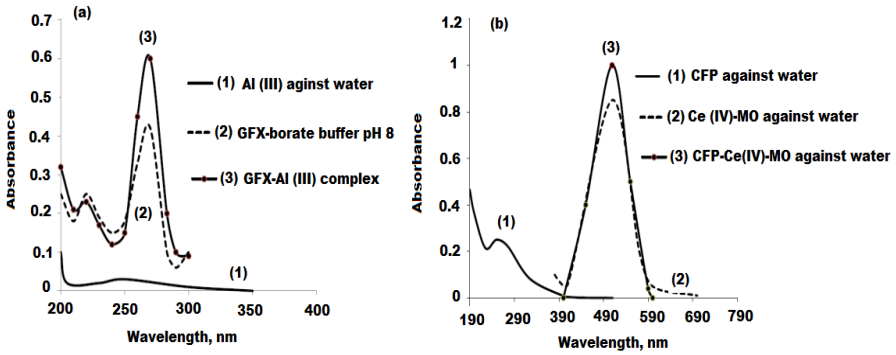


Figure 2: Absorption spectra of (a) GFX and (b) CFP: CFP (7.0 µgmL^{-1}), Ce(IV) (2.0 µg mL^{-1}) and MO (20 µg mL^{-1}).

The spectra showed that, no absorption peak was noticed for the aqueous solution of $AlCl_3$ (Figure 2a-1), the appearance of one absorption peak for GFX at 270 nm (Figure 2a-2) and a high intensity peak at 270 nm for GFX-Al (III) complex (Figure 2a-3). The observed maximum peak of the formed complex is due to the strong acidity of Al^{3+} that can bind with the carbonyl and carboxylic oxygen of the tested drug. [26]

In the determination of CFP (method C), the ability of cerium sulfate to oxidize CFP and interact with MO is the basis of the indirect spectrophotometric determination of the drug. The developed method was based on the reaction between excess cerium sulfate with CFP in an acidic medium and the unreacted oxidizing agent reacts with excess MO and the residual of MO was estimated by deliberating its absorbance at 509 nm. The absorbance was gradually increased with increasing CFP concentration (Figure 2b)**.** The aqueous solution of CFP showed two absorption peaks at 190 and 240 nm (Figure 2b-1), while that of cerium sulfate and MO solution exhibit absorption at 509 nm (Figure 2b-2). However, the addition of aqueous cerium sulfate to the solution of CFP resulted in a high intensity peak at 509 nm (Figure 2b-3).

Optimal conditions of method A and B: The pH ranges (3.9 -5) and (6-12) of acetate and borate buffers were applied to study the influence of pH on the complex formation. The maximum absorbance was attained at pH 8. It was noticed that a sharp decrease in the absorbance was observed at high pH (Figure 3a). The influence of buffer type on the reaction was studied at pH 8 using various buffer solutions including, Britton Robinson, phosphate and tris and borate buffers. The use of borate buffer gave the highest absorbance (Figure 3b). Therefore, it was selected for further investigations.

The influence of the borate buffer (pH 8) volume on the absorbance intensity of the resulted complex was investigated. The increase of borate buffer (pH 8) causes an elevation in the absorbance value of the formed complex up to 1.5 mL and then it remained constant. Accordingly, 2.0 mL of borate buffer was used during this study (Figure 3c). Also, the increase of $A1Cl_3$ (1.0×10^{-1} mol L^{-1}) volume causes a gradual elevation in the absorbance value of the complex up 1.5 mL, then it displayed constant values, so that 2.0 mL of 1.0×10^{-1} mol L^{-1} $A1Cl_3$ was chosen for the study (Figure 3d).

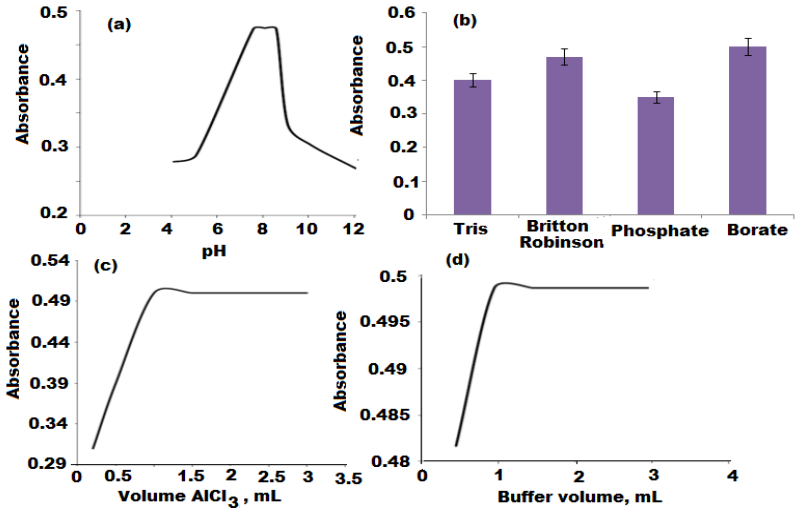


Figure 3: Optimal conditions of methods A and B: (a) Effect of pH, (b) Effect of buffer type, (c) Effect of $AlCl_3$ volume and (d) Effect of buffer volume.

Optimization of method C conditions: The influence of MO concentration on the reaction product absorbance was tested at 509 nm using different volumes (0.5-3 mL) of 200 μgmL^{-1} MO solution. (Figure 4a) showed that the maximum absorbance was achieved by using 1.0 mL, above this amount it decreased.

The influence of $CeSO_4$ concentration on the reaction product absorbance was investigated at 509 nm using the volume range (0.2-2.0 mL) of 500 μgmL^{-1} $CeSO_4$ solution. Maximum absorbance was achieved by using 1.0 mL above this amount it decreased (Figure 4b).

The analytical reaction was performed in sulfuric acid medium. The high absorption intensity was obtained by using 1.0 mL of 5.0 mol L^{-1} H_2SO_4 for CFP (Figure 4c).

It was noticed that the immediate addition of MO to the acidic solution containing the drug and cerium sulfate, a very low absorbance was resulted. This can be attributed to the oxidation of the drug by cerium (IV) sulfate is a time dependent reaction. The results displayed an increase in the absorbance up to 40 min, then remaining constant (Figure 4d). Therefore, a reaction time of 40 minutes was used for CFP determination. Certain time 5 min was required for bleaching the dye color by the residual oxidant. The stability of the formed reaction product color was remained for hours.

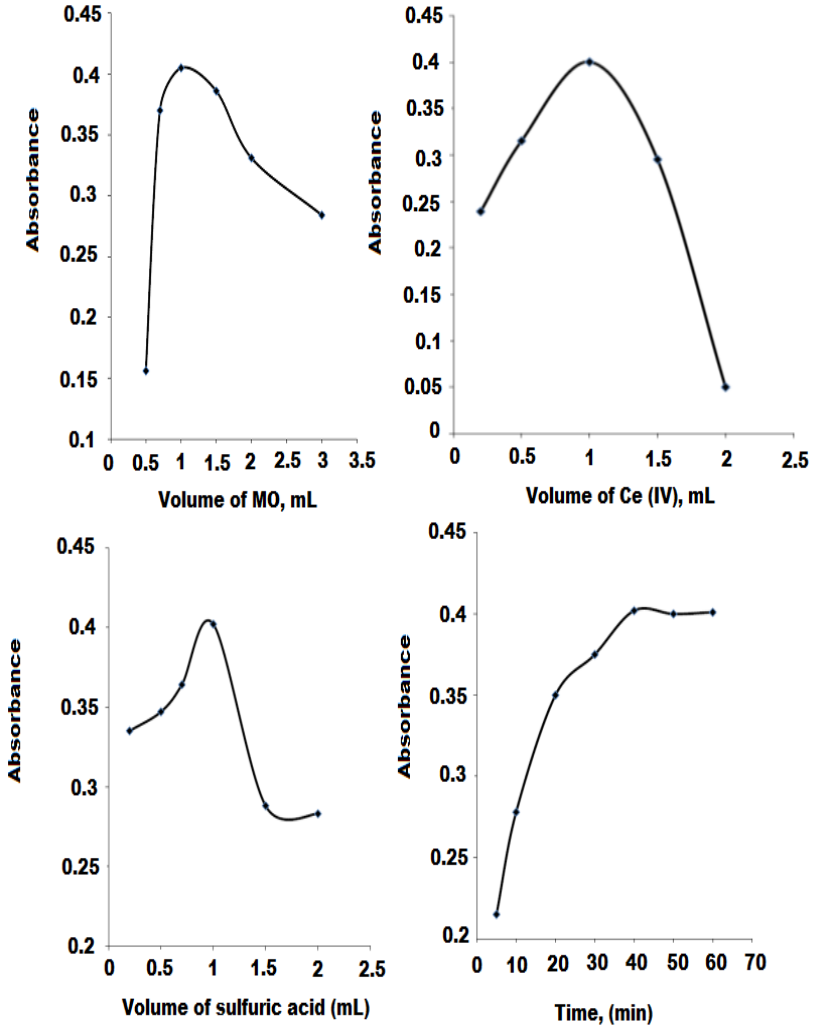
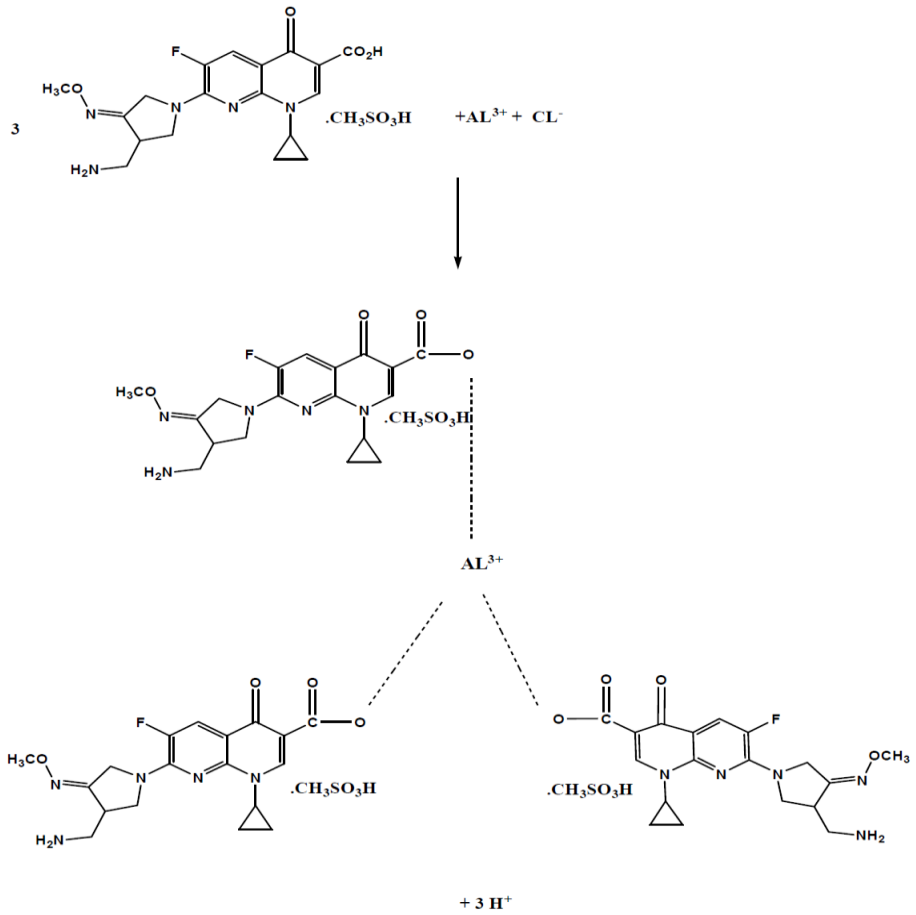


Figure 4: Optimal conditions of method C: (a) Effect of MO volume, mL, (b) Effect of Ce (IV) volume, mL (c) Effect of sulfuric acid volume, mL and (d) Effect of reaction time.

Stoichiometry and reaction mechanism: The molar ratio method,[29] was employed to study the stoichiometry of the reaction. It was addressed 1:3 ratio (GFX: Al^{3+}) (Figure 5a). According to this ratio, the reaction pathway was illustrated in Scheme 1.



Scheme 1: The proposed pathway for the reaction of GFX with Al^{3+} in borate buffer

The stoichiometry of the reaction was studied adopting the limiting logarithmic method. [30] The plotting of log absorbance vs. log ($CeSO_4$) and log (CFP) gave straight lines, with slope values of 0.953 and 0.95, respectively (Figure 5b). Therefore, the reaction proceeds in a ratio of 1: 1 ($CeSO_4$ to CFP). The reaction pathway was illustrated in Scheme 2.

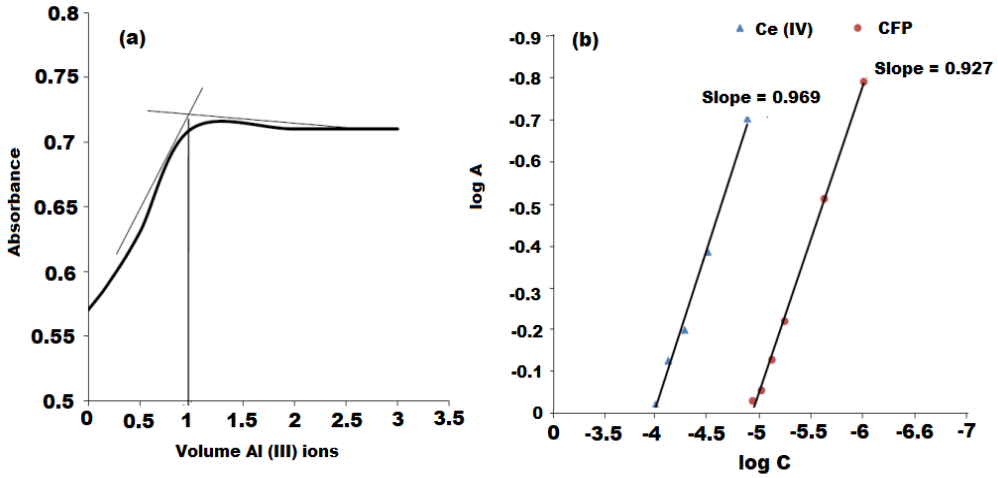


Figure 5: (a) molar ratio method of complex GFX and Al, $C_{Al}^{3+} = C_{GFX} = 5\times10^{-5}$ mol L^{-1} and (b) Limithmitric plots of molar ratio reaction of CFP with $CeSO_4$

Method validation: Under described experimental conditions, the calibration graphs of GFX and CFP using $AlCl_3$ and cerium (IV) sulfate, respectively, by analytical methods A, B and C were plotted over the concentration ranges of 1-10, 2-24 and 2-14 µg mL^{-1} for the three methods as illustrated in Figures 6a-c, respectively.

The data recorded including, the regression equations and correlation coefficients were presented in Table 1. The obtained data gave the following regression equations: A = 0.099C+0.00002, ΔA = 0.033C + 0.042 and 0.0203C + 0.0736 with correlation coefficients r= 0.9999 for the three methods respectively. Where: (A) is the absorbance at 270 nm, (ΔA) is the absorption difference between drug and complex (C) is the concentration of the drug in µg mL^{-1}.

The limits of quantification (LOQ) detection (LOD) [31] were determined using the following equations: LOQ= 10 Sa/b and LOD=3.3 Sa/b. Where: (Sa) is the standard deviation of the intercept and b is the slope of the calibration graph. LOQ values were as 0.281, 0.577 and 0.533 µg mL^{-1} while LOD values were 0.093, 0.191 and 0.176 µg mL^{-1} for the three spectrophotometric approaches, respectively.

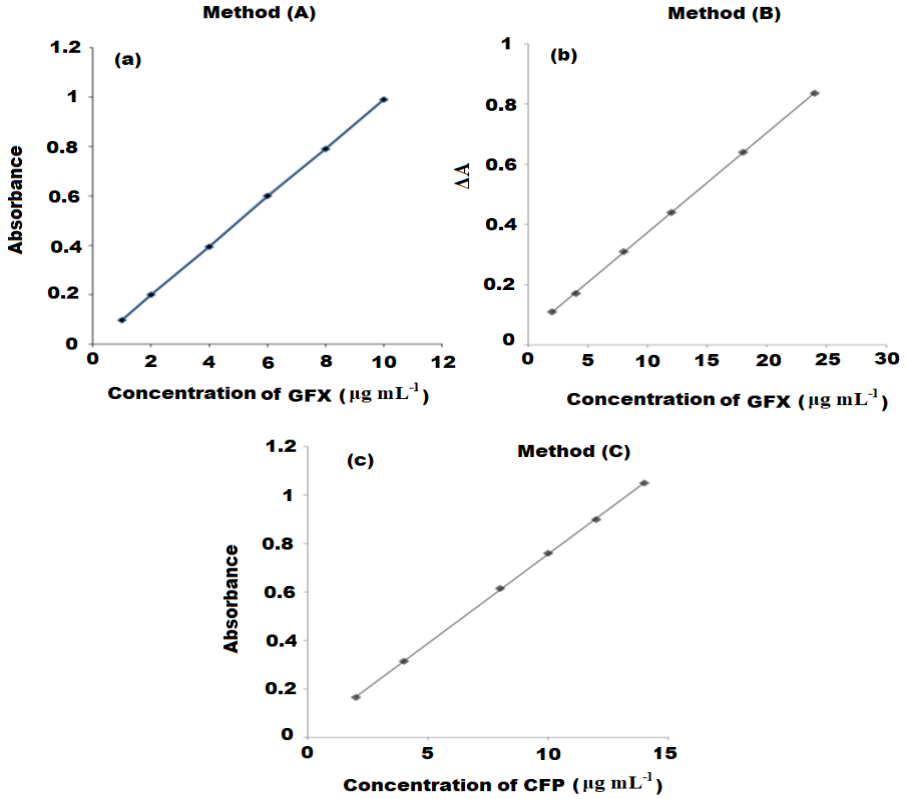


Figure 6: Calibration graphs for spectrophotometric determination of GFX and CFP using $AlCl_3$ and $CeSO_4$, respectively

Table 1: Performance Data for the proposed spectrophotometric methods using $AlCl_3$.

Parameter	Method A	Method B	Method C
Concentration rang (µg ml^{-1})	1-10	2-24	2-14
Correlation coefficient (r)	0.9999	0.9999	0.9999
Intercept	2×10^{-5}	0.0416	0.0736
Slope	0.09917	0.033	0.0203
Sa[a]	0.00278	0.00191	0.0039
Sb[b]	0.00046	0.00014	0.00042
LOD (µg mL^{-1})[c]	0.0925	0.1905	0.176
LOQ (µg mL^{-1})[d]	0.281	0.577	0.533

[a] S_a is the standard deviation of the intercept. [b] S_b is the standard deviation of the slope. [c] LOD is the limit of detection. [d] LOQ is the limit of quantification

The % error was calculated using 1-10, 2-24 and 2-14 µg mL^{-1} of the tested drugs to ensure the accuracy of the developed spectrophotometric methods. The outcomes were -1 to 0.5, -0.5 to 1.5 % at 270 nm and -1 to 0.635 µg mL^{-1} at 509 nm for the three spectrophotometric methods, respectively. The results obtained from the quantification of GFX and CFP in their bulk powders were compared with those of published spectrophotometric methods,[14,16] respectively. Statistical assessment using Student's t-test and variance ratio F-test,[32] indicated excellent accuracy of the above previously mentioned methods, respectively (Tables 2 and 3).

Table 2: Spectrophotometric analysis of the pure form of GFX in the presence of $AlCl_3$ using methods A and B in comparison with reported method.[14]

Taken µg mL^{-1}	Found µg mL^{-1}	Error %	Recovery %		Taken µg mL^{-1}	Found µg mL^{-1}	Error %	Recovery %	
			Method A	Published Method[14]				Method B	Published Method [14]
1.0	0.99	-1	98.80	--	2.0	2.03	1	101.33	
2.0	2.01	0.5	100.32	99.94	4.0	4.01	0.25	100.29	99.94
4.0	4.00	0.075	100.08	98.00	12.0	11.98	--	99.85	98.00
6.0	0.02	0.33	100.50	99.75	18.0	17.99	-0.17	99.98	99.75
8.0	7.99	-0.125	99.95	99.60	24.0	23.89	-0.03	99.55	99.60
10.0	9.99	-0.1	99.93				-0.46		
Mean±SD			99.93±0.6	99.23±1.07	100.36±0.7				99.23±1.07
t-test			1.17(2.365)*		1.84(2.365)*				
F-test			2.57(6.59)*		2.53(6.59)*				

**The Figures between parentheses are the tabulated values of t- and F- tests at p = 0.05*[32]

Table 3: Spectrophotometric analysis of CFP in pure using cerium sulfate in comparison with reported method.

Taken µg mL^{-1}	Found µg mL^{-1}	Error %	Recovery %	
			Method A	Published Method [16]
2.0	1.98	-1	99.0	99.85
4.0	3.99	-0.25	99.7	99.93
8.0	8.04	0.635	100.50	99.50
10.0	9.90	-1	99.00	99.63
12.0	11.93	-0.583	99.42	99.06
14.0	13.97	-0.214	99.79	99.28
Mean±SD			99.93±0.6	99.59±0.3
t-test			0.217(2.228)*	
F-test			3.314(5.05)*	

**The Figures between parentheses are the tabulated values of t- and F- tests at p = 0.05* [32]

Further, study was carried out using intra-day and inter-day assay to confirm the precision of the developed methods. The intermediate precision of the suggested analytical methods was expressed as relative standard deviations (Table 4).

Table 4: Intra-day and inter-day assay for intermediate precision of the three spectrophotometric methods.

	GFX						CFP		
	Method A (µg mL^{-1})			Method B (µg mL^{-1})			Method C (µg mL^{-1})		
	1.0	4.0	10.0	4.0	12.0	24.0	2.0	8.0	14.0
Intra-day	98.80	100.08	99.93	100.29	99.85	99.55	99.16	99.47	99.10
	99.01	99.78	100.06	99.51	99.56	100.03	99.00	100.50	99.79
	98.66	100.12	100.52	100.69	98.47	99.79	99.44	100.09	99.82
Mean ±SD	98.91 ±0.1	99.93 ±0.2	99.99 ±0.1	99.90 ±0.6	99.71 ±0.2	99.79 ±0.3	99.08 ±0.11	99.99 ±0.7	99.46 ±0.5
Inter-day	99.32	99.84	99.95	98.77	99.16	100.06	99.28	100.6	99.06
	99.70	99.40	99.61	99.40	99.73	99.45	99.66	101.03	99.86
	98.27	98.87	98.25	98.25	100.47	99.89	99.91	99.56	99.57
Mean ±SD	99.51 ±0.3	99.62 ±0.3	99.78 ±0.2	99.19 ±0.3	99.45 ±0.4	99.67 ±0.3	99.47 ±0.3	100.84 ±0.3	99.46 ±0.6

The selectivity of the analytical procedure for the estimation of GFX and CFP was studied in the presence of the some possible additives present in (Factive®320 mg/tablet) and (Meiact®200 mg/tablet) e.g. talc, magnesium stearate, titanium dioxide, microcrystalline cellulose, polyethylene glycol and povidone. No significant interference was found. Therefore, the developed spectrophotometric methods can be considered for determination of GFX and CFP.

To study the suitability of the suggested spectrophotometric methods, GFX and CFP were determined in their (Factive®320 mg/tablet) and (Meiact®200 mg/tablet) tablets. Different concentrations of the selected drugs were analyzed and the percentage

recoveries were calculated as a result of three replicate determinations. Good agreement was noticed between the outcomes and those obtained by the published spectrophotometric methods.[14,16] The statistical comparison showed no significant differences were recorded (Table 5).

Table 5: Spectrophotometric analysis of GFX and CFP using $AlCl_3$ (method A and B) and $CeSO_4$ (Method C) in comparison with reported methods.[14,16]

Taken µg mL^{-1}	Recovery % Method A	Taken µg mL^{-1}	Recovery % Method B	Published Method[14]	Taken µg mL^{-1}	Recovery % Method C	Published Method [16]
2.0	99.51	2.0	100.25	99.30	2.0	99.05	100.14
4.0	99.84	8.0	99.38	99.70	4.0	99.79	99.67
6.0	100.69	14.0	99.63	99.10	8.0	100.15	99.35
8.0	99.68	20.0	100.69	98.80	10.0	99.96	99.90
10.0	99.25	24.0	99.56	99.85	12.0	99.37	99.57
				99.60	14.0	99.63	99.50
Mean±SD	99.93±0.5		99.99±0.6	99.35±0.4	99.66±0.4		99.73±0.3
t-test	1.98 (2.262)*		1.97(2.262)*		0.135 (2.228)*		
F-test	1.49(5.19)*		1.91(5.19)*		2.16 (5.05)*		

**The Figures between parentheses are the tabulated values of t- and F- tests at p = 0.05*[32]

CONCLUSIONS

Successful new and very sensitive spectroscopic approaches were developed for quantification of GFX and CFP in their bulk powders and dosage forms using aluminum chloride and cerium (IV) sulfate, respectively. The suggested procedures are more sensitive and selective rather than the previously addressed spectrophotometric approaches for the estimation of GFX and CFP in term of their simplicity, no preconditioning steps, and inexpensive analytical reagents and provide high recoveries. The proposed methods also can be applied to the industrial and drug quality control without any interference can be caused by the possible additives of tablets.

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