

# Spectrofluorimetric determination of cefixime using terbium-danofloxacin probe

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## ABSTRACT

**Objective(s):** Cefixime (Cfx), is a semi-synthetic third-generation oral cephalosporin antibiotic that is prescribed for the treatment of susceptible infections. There are some procedures for the determination of Cfx in pharmaceutical formulations and biological samples. Herein a spectrofluorimetric method was proposed for Cfx determination based on the fluorescence quenching of terbium-danofloxacin (Tb<sup>3+</sup>-Dano) in the presence of Cfx.

**Materials and Methods:** Cfx was detected based on fluorescence quenching of terbium-danofloxacin (Tb<sup>3+</sup>-Dano) in the presence of Cfx with maximum excitation and emission wavelengths at 347 nm and 545 nm, respectively. The quenched fluorescence intensity of Tb<sup>3+</sup>-Dano system is proportional to the concentration of Cfx. The optimum conditions for the determination of Cfx were studied.

**Results:** The maximum response was achieved under optimum conditions of [Tris buffer]= 0.008 mol/l (pH 6.5), [Tb<sup>3+</sup>]=1×10<sup>-4</sup> mol/l and [Dano]=1×10<sup>-4</sup> mol/l. The developed method was evaluated in terms of accuracy, precision and limit of detection. The linear concentration ranges for quantification of Cfx were 8.8×10<sup>-8</sup>-8.8×10<sup>-7</sup> mol/l and 1.1×10<sup>-7</sup>-8.8×10<sup>-7</sup> mol/l in standard and human serum samples with the detection limits (S/N=3) of 2.8×10<sup>-8</sup> mol/l and 3.9×10<sup>-8</sup> mol/l, respectively. The Cfx was determined in pharmaceutical tablets and spiked serum samples and the results were satisfactory.

**Conclusion:** This method is simple, practical and relatively interference-free for determination of Cfx in pharmaceutical tablets and serum samples.

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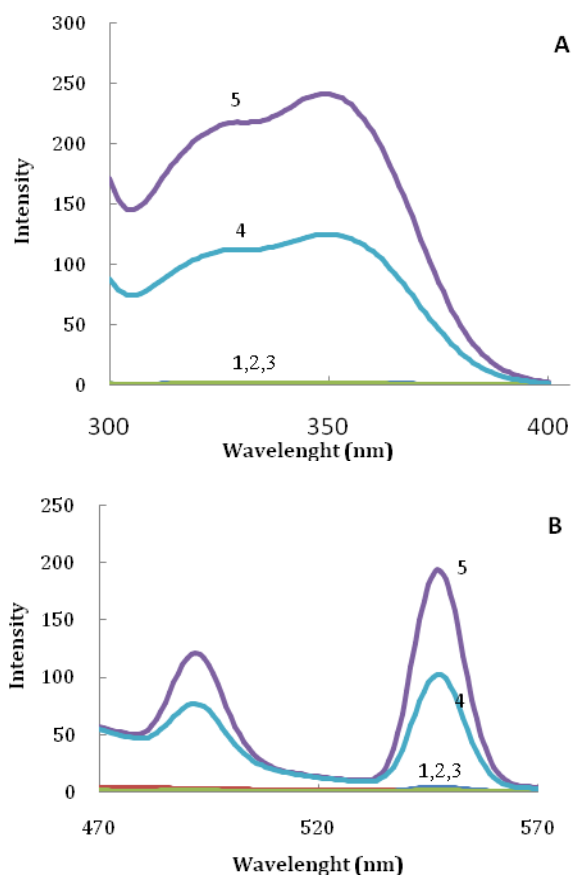
## Introduction

Cefixime, ((6R,7R)-7-[(Z)-2-(2-amino-4-thiazolyl)-2-(carboxymethoxyimino) acetamido]-8-oxo-3-vinyl-5-thia-1-azabicyclo-[4.2.0]-oct-2-ene-2-carboxylic acid), is a semi-synthetic third-generation oral cephalosporin antibiotic being prescribed for the treatment of susceptible infections such as gonorrhoea, otitis media, pharyngitis, lower respiratory tract infections like bronchitis, and urinary tract infections (1-3). There are some procedures for the determination of cefixime (Cfx) in pharmaceutical formulations and biological samples including spectrophotometry (4-6), high performance liquid chromatography (7), high performance thin layer chromatography (HPTLC) (8) and electrochemical methods (9-11). A few spectrofluorimetric methods have been reported for determination of Cfx based on the following processes: Cfx oxidation in the presence of Ce (IV) and indirect determination of Cfx through

measurement of fluorescence active Ce (III) ion (12); quenching of the fluorescence intensities of terbium (III) -Tris base and calcein systems as a fluorescence probe in the presence of Cfx (13,14); the reaction between cephalosporine and 1,2-naphthoquinon-4-sulfonic to give highly fluorescent derivatives extracted with chloroform and measurement of fluorescence intensity (15); and the reaction between Cfx and 2-cyanoacetamide in the presence of 21% ammonia at 100 °C (16). These methods have the downsides of interferences with other compounds, require expensive reagents, or possess narrow range of calibration curves.

One of the most important luminescence probes is lanthanide ions complexes that have attracted wide attention. Because of some luminescence characteristics of rare earth ions, such as narrow spectral width, long luminescence lifetime, large Stokes shift and strong binding with biological

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**Figure 1.** Excitation and emission spectra: (A): excitation spectra and (B): emission spectra of (1)  $Tb^{3+}$ , (2) Cfx, (3)  $Tb^{3+}$ -Cfx, (4)  $Tb^{3+}$ -Dano-Cfx, (5)  $Tb^{3+}$ -Dano  
 Experimental condition:  $[Tb^{3+}]$ :  $1 \times 10^{-4}$  mol/l,  $[Dano]$ :  $1 \times 10^{-4}$  mol/l,  $[Cfx]$ :  $2.2 \times 10^{-6}$  mol/l, pH=6.5,  $\lambda_{ex}/\lambda_{em}$ =347 nm / 545 nm

molecules, they are widely used as fluorescent probes. In particular, attentions have been directed towards two rare earth cations,  $Tb^{3+}$  and  $Eu^{3+}$  (17).

In this work, the fluorescence of terbium-danofloxacin ( $Tb^{3+}$ -Dano) complex is used as a probe for determination of Cfx in pharmaceutical tablets and spiked human serum samples. Danofloxacin (Dano) is one of the third-generation of quinolone antibiotics. Dano could form coordination complex with terbium, which emits the characteristic fluorescence of terbium (18). The fluorescence intensities of  $Tb^{3+}$ -Dano complex are greatly quenched when Cfx is added. Under optimal conditions, the enhanced fluorescence intensity is proportional to the concentration of Cfx. It was found that the fluorescence probe of  $Tb^{3+}$ -Dano complex had a high sensitivity and wide range for Cfx determination at ng levels. In addition, the proposed fluorescence probe is inexpensive and the reagents are easily available and environmentally friendly. The  $Tb^{3+}$ -Dano complex has good stability and solubility in water and does not require the addition of luminescence enhancers.

## Materials and Methods

### Chemicals and reagents

Analytical-grade ethanol, methanol, 2-propanol, hydrochloric acid (HCl), acetonitrile, and tris-[hydroxymethyl] aminomethan (Tris) were obtained from Merck (Germany). Terbium (III) chloride hexahydrate ( $TbCl_3 \cdot 6H_2O$ ) was from Acrose organics (Fairlawn, New Jersey, United States), Dano powder from Jamedat Afagh pharmaceutical company (Semnan, Iran), and Cfx powder was from Daana pharmaceutical company (Tabriz, Iran). Double distilled water being prepared using Millipore-Q-Plus water purification system (Millipore, Bedford, MA, USA) was used in this study.

### Sample preparation

#### Tablet treatment

Five 200 mg labeled-tablets of Cfx were weighed and ground to a fine powder using a pestle and mortar. Amount of 0.200 g aliquot of the homogenized powder was dissolved in 80 ml methanol, then filtered through an ordinary filter paper and diluted to the mark in a 100 ml calibrated flask. The solution was diluted with double distilled water and convenient aliquots from the diluted solution were taken for the determination of Cfx using the proposed procedure.

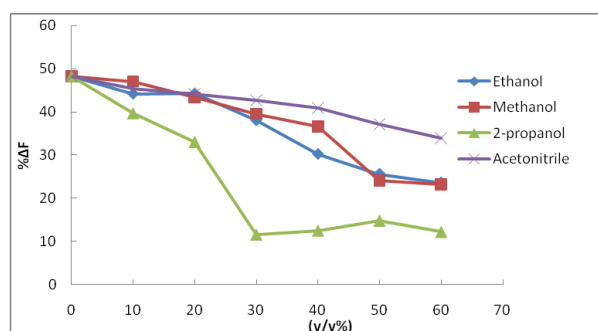
#### Serum treatment

Serum was spiked with convenient amounts of Cfx stock solution. A volume of 1.5 ml of acetonitrile was added to 0.5 ml of spiked serum and centrifuged for 10 min at 10,000 rpm. Subsequently, 1 ml of the supernatant solution was diluted to the final volume of 2.5 ml, and then 1 ml of the prepared sample was added to the reagents mixture to determine the amount of Cfx using the developed method (19).

#### Method of analysis

A  $2.2 \times 10^{-3}$  mol/l stock solution of Cfx was prepared by dissolving 0.100 g of Cfx powder in 100 ml methanol. A  $1 \times 10^{-2}$  mol/l solution of  $Tb^{3+}$  was prepared by dissolving 0.373 g of  $TbCl_3 \cdot 6H_2O$  powder in 100 ml double distilled water and stored in polyethylene container to avoid memory effects of terbium adsorbed to glass vessels. A  $1 \times 10^{-2}$  mol/l stock solution of Dano was prepared by dissolving 0.357 g of Dano powder in double distilled water. Working solutions of Cfx, Dano and  $Tb^{3+}$  were obtained by appropriate dilution of their stock solutions with double distilled water. Tris-hydrochloric acid (Tris-HCl) buffer solution (0.01 mol/l) was prepared by dissolving 1.210 g of Tris-base in 100 ml water, adjusting the pH with 1 mol/l HCl solution.

In order for Cfx analysis in different samples (standard, tablet and spiked human serum), reagents were added in a 10 ml test tube in the following order (in the absence and presence of Cfx): 800  $\mu$ l of



**Figure 2.** Solvent effect on the quenched fluorescence intensity ( $\Delta F\%$ )

0.1 mol/l Tris-HCl buffer (pH 6.5), 1 ml of  $1 \times 10^{-3}$  mol/l  $Tb^{3+}$  solution, 1 ml of  $1 \times 10^{-3}$  mol/l Dano solution and 1 ml aliquots of sample solutions were pipetted into reagents (Tris.HCl buffer - $Tb^{3+}$ - Dano-Cfx). A volume of this mixture was diluted to final volume of 10 ml with double distilled water (the Cfx concentration ranges were  $8.8 \times 10^{-8}$ - $8.8 \times 10^{-7}$  mol/l) and after 3 min, the luminescence intensity (F) was measured in 1 cm quartz cell with an excitation wavelength of  $\lambda_{ex} = 347$  nm and an emission wavelength of  $\lambda_{em} = 545$  nm. All measurements were performed at  $25^\circ C$ . The quenched fluorescence intensities of  $Tb^{3+}$ -Dano by Cfx were represented as  $\Delta F\% = 100 (F_0 - F)/F_0$ , where F and  $F_0$  are the fluorescence intensity of the probe ( $Tb^{3+}$ -Dano) with and without Cfx, respectively.

### Instruments

All fluorescence spectra were recorded using a Jasco FP-750 spectrofluorimeter (Kyoto, Japan) equipped with a 150 W xenon lamp using a 1 cm quartz cell. The excitation and emission monochromator bandwidths were 5 nm. All measurements were performed at  $25 \pm 0.1^\circ C$  that was controlled using a peltier thermostated cell holder (Jasco, Japan). The pH of solutions was measured with Metrohm 654 pH meter (Herisau, Switzerland).

## Results

### Fluorescence spectra

Fluorescence emission and excitation spectra of  $Tb^{3+}$  (1), Cfx (2),  $Tb^{3+}$ -Cfx (3),  $Tb^{3+}$ -Dano-Cfx (4) and  $Tb^{3+}$ -Dano (5) are shown in Figure 1.

**Table 1.** Intra assay precision and accuracy of calibration standards for cefixime (Cfx)

	Nominal concentration (mol l <sup>-1</sup> × 10 <sup>-7</sup> ) (N=3)	Found concentration (mol l <sup>-1</sup> × 10 <sup>-7</sup> ) (N=3)	Precision (RSD %)	Accuracy (RE %)
Cfx	1.32	1.29	3.5	-2.2
	2.20	2.17	3.6	-1.3
	3.30	3.32	3.1	0.4
	4.41	4.48	3.2	1.5
	8.82	8.9	2.5	1.3
Cfx in spiked human serum	1.10	1.15	6.7	4.7
	1.65	1.71	6.5	3.1
	2.20	2.27	6.1	3.2
	4.41	4.37	4.3	-0.8
	8.82	8.99	3.1	1.9

### Optimization of experimental conditions

#### Effect of pH

Fluorescence intensity of a series of 0.01 mol/l Tris buffer solutions with the pH ranging from 5.0 to 8.5 were measured at  $\lambda_{ex}/\lambda_{em} = 347\text{nm}/545\text{nm}$  (Figure 2). Concerning the results, pH = 6.5 was selected for further investigations.

#### Effect of buffer concentration

The influence of Tris buffer (pH=6.5) concentration was studied. The results indicated that  $\Delta F\%$  of the probe achieved the maximum value when the buffer concentration was within the range of 0.006 mol/l to 0.01 mol/l. Therefore, 800  $\mu$ l of 0.01 mol/l Tris-HCl in 10 ml mixture was the optimum buffer volume.

#### Effect of Dano concentration

The influence of Dano concentration on the fluorescence intensities was studied and it was found that the quenched fluorescence intensity of  $Tb^{3+}$ -Dano-Cfx system had reached to maximum when the concentration of Dano was  $1 \times 10^{-4}$  mol/l. Hence, a concentration of  $1 \times 10^{-4}$  mol/l was used as optimum concentration of Dano for further studies.

#### Effect of $Tb^{3+}$ concentration

Effect of  $Tb^{3+}$  concentration on the decrease in fluorescence intensity of  $Tb^{3+}$ -Dano-Cfx was studied at constant concentrations of  $2.2 \times 10^{-6}$  mol/l of Cfx. The  $\Delta F\%$  was the highest when the  $Tb^{3+}$  concentration in the mixture was in the range of  $1 \times 10^{-4}$  mol/l -  $5 \times 10^{-5}$  mol/l. The  $Tb^{3+}$  concentration of  $1 \times 10^{-4}$  mol/l was selected for further analysis.

#### Effect of reaction time

Under optimum conditions, the effect of time on the fluorescence intensity was investigated. The results showed that the fluorescence intensity is stable for 60 min after all the reagents are added. This is due to the rapid complex formation between  $Tb^{3+}$ , Dano and Cfx. In this study, 3 min was set as the standard time interval for all fluorescence intensity measurements. In other word, the reaction time was 3 min and the ternary complex was stable for 60 min.

**Table 2.** Assay precision and accuracy of quality control samples

	Nominal concentration (mol l <sup>-1</sup> × 10 <sup>-7</sup> )	Intra-assay (within-day) precision (RSD%) (N=5)	Inter-assay (between-day) precision (RSD %) (N=3)	Accuracy (RE %)
Cefixime (Cfx)	1.32	3.19	4.40	-1.83
	4.41	2.98	4.25	-1.20
	6.61	2.75	3.98	0.73
Cfx in spiked human serum	1.54	7.01	10.24	3.16
	3.30	6.58	9.68	-3.51
	6.61	5.06	8.91	-2.04

**Effect of temperature**

Another parameter influencing the fluorescence intensity is temperature. The effect of temperature on the quenched fluorescence intensity was investigated and 25°C was selected for further studies.

**Effect of addition order of reagents**

The experiments showed that addition order of reagents affects the fluorescence intensity of probe. To study this, series of solutions were prepared with different addition sequence and the fluorescence intensities were measured. It was found that there was a negligible difference between  $\Delta F\%$  amounts of addition sequences. Thus, the addition order of Tris buffer-Tb<sup>3+</sup>-Dano-Cfx was selected as our addition order.

**Effect of surfactant**

In order to improve fluorescence intensity, various surfactants such as CTAB, Tween 80, SDBS, SDS, Triton X-100 and Triton X-114 were added to the solution and their effects were studied. None of the studied surfactants had significant effect on  $\Delta F\%$ .

**Solvents effect**

Under optimum conditions, the effect of organic solvents such as methanol, ethanol, 2-propanol and acetonitrile in the range of 0-60 % (v/v) was studied. The results indicated that increasing the volume of organic solvents could decrease the fluorescence intensity ( $\% \Delta F$ ).

**Interference studies**

Under proper conditions, the effects of interferences of coexisting substances on the quenched fluorescence intensity were tested. The results are shown in Table 1.

**Analytical application and assay validation****The calibration graphs and detection of limits**

The calibration graph for Cfx was obtained under optimum conditions (Table 2). There was a good linear relationship between the quenched fluorescence intensities and the concentrations of Cfx in a wide range of  $8.8 \times 10^{-8}$ - $8.8 \times 10^{-7}$  mol/l in the standard sample, and  $1.1 \times 10^{-7}$ - $8.8 \times 10^{-7}$  mol/l in serum samples. The detection limits for Cfx in standard and serum samples (S/N=3) were  $2.8 \times 10^{-8}$  mol/l and  $3.9 \times 10^{-8}$  mol/l, respectively. The correlation coefficient was 0.99 for both samples.

**Precision and accuracy**

The results of intra-assay precision and accuracy of calibration standards are shown in Table 1. All relative standard deviations (RSD%) were below 5% for standard samples and below 15% for serum samples, which were acceptable for bioanalytical methods according to FDA recommendations (22). Inter- and intra-assay precisions along with accuracy for quality control samples are listed in Table 2.

**Recovery**

The recoveries for the investigated Cfx samples are summarized in Table 3. The mean recoveries for Cfx in serum were 103.1%, 96.5 % and 97.9 %, respectively.

**Analytical applications**

The proposed method was applied for the determination of Cfx in pharmaceutical tablets. Each Cfx tablet contained 200 mg and by employing the proposed method, the measured amount was 202.1 mg. Therefore, the mean recovery for determination of Cfx was 101.05 % with an RSD of 2.7%.

**Table 3.** Absolute and mean recoveries for cefixime (Cfx)

	Nominal concentration (mol l <sup>-1</sup> × 10 <sup>-7</sup> )	Found concentration (mol l <sup>-1</sup> × 10 <sup>-7</sup> ) (N=5)	Recovery %	Mean Recovery %	Precision of recovery (RE%)
Cfx	1.32	1.29	98.1	99.2	-1.9
	4.41	4.35	98.8		-1.2
	6.61	6.66	100.7		0.7
Cfx in spiked human serum	1.54	1.59	103.1	99.1	3.1
	3.30	3.19	96.5		-3.5
	6.61	6.48	97.9		2.1

**Table 4.** Determination of cefixime (Cfx) in spiked human serum sample

Nominal concentration (mol l <sup>-1</sup> × 10 <sup>-7</sup> )	Found concentration using developed method (mol l <sup>-1</sup> × 10 <sup>-7</sup> )	RSD% (N=3)	Recovery %
2.20	2.27	7.49	103
6.61	6.48	6.84	98

The method was also applied for the determination of Cfx in spiked human serum samples which their results are shown in Table 4. The RSD% values (less than 15.0%) were achieved for the samples which is acceptable for biological samples according to FDA guidelines (21).

The proposed method has a broad linear range and 14-fold lower LOD than the previous developed method (13) using Tb<sup>3+</sup> without danofloxacin. In addition, our method was developed for determination of Cfx in serum samples.

## Discussion

The complex of Tb<sup>3+</sup>-Dano displays an excitation peak at 347 nm and two emission peaks at around 490 nm and 545 nm, which corresponds to the transmission of <sup>5</sup>D<sub>4</sub> level of terbium to the <sup>7</sup>F<sub>6</sub> and <sup>7</sup>F<sub>5</sub> levels, respectively (17). Since the emission intensity at 545 nm was stronger than that at 490 nm, the excitation and emission peaks were set at 347 nm and 545 nm, respectively. Tb<sup>3+</sup>, Cfx and Tb<sup>3+</sup>-Cfx do not show the characteristic fluorescence spectra. The fluorescence of the Tb<sup>3+</sup>-Dano-Cfx complex was similar to that of Tb<sup>3+</sup>-Dano. However, the fluorescence intensity of Tb<sup>3+</sup>-Dano complex was quenched by Cfx which indicated that there was an

interaction between Cfx and Tb<sup>3+</sup>-Dano complex. The complementary experiments showed that the quenched fluorescence intensity was proportional to the concentration of Cfx.

Experimental results indicated that the maximum fluorescence intensity of system was reached at pH 6.5. Therefore, pH 6.5 was selected for further research. The Tris-HCl was used as a buffer for pH adjustments of the solutions due to its good characteristics such as not quenching the fluorescence of the probe, good stability and having a wide range of pH (20, 21).

Effect of pH on the quenched fluorescence intensity of Tb<sup>3+</sup>-Dano system ( $\Delta F\% = [(F_0 - F)/F_0] \times 100$ , in which F and F<sub>0</sub> were the fluorescence intensities of the probe with and without Cfx), was studied within the range of 0.005 mol/l - 0.03 mol/l of Tris buffer solutions at constant values of Tb<sup>3+</sup>, Dano, Cfx at 1 × 10<sup>-4</sup> mol/l, 1 × 10<sup>-4</sup> mol/l, 2.2 × 10<sup>-6</sup> mol/l, respectively.

The influences of Dano and Tb<sup>3+</sup> concentrations on the quenched fluorescence intensity were studied to the next step. The effect of reaction time was investigated under optimal conditions such as the buffer pH as well as concentrations of buffer, Dano and Tb<sup>3+</sup>. The results showed that the fluorescence

**Table 5.** Analytical characteristics of available methods for determination of cefixime (Cfx)

Method	Linear range	LOD	LOQ	Application	Ref
Spectrophotometric method ( flow injection analysis)	0.08-2.00 mg ml <sup>-1</sup>	60 µg ml <sup>-1</sup>	200 µg ml <sup>-1</sup>	drug formulation	(4)
High performance liquid chromatography method	0.004-5.00 µg ml <sup>-1</sup>	1 ng ml <sup>-1</sup>	4 ng ml <sup>-1</sup>	human plasma	(7)
High performance thin layer chromatography method	125-500 ng	18.4 ng	61.33 ng	dosage form	(8)
electrochemical method (Voltammetric analysis)	50 ng ml <sup>-1</sup> -25.6 µg ml <sup>-1</sup>	3.99 ng ml <sup>-1</sup> (by SWCAdSV)	13.3 ng ml <sup>-1</sup> (by SWCAdSV)	tablet	(9)
		7.98 ng ml <sup>-1</sup> (by DPCAdSV)	26.6 ng ml <sup>-1</sup> (by DPCAdSV)	urine	
		1-60 µg ml <sup>-1</sup>	12.6 ng ml <sup>-1</sup> (by SWCAdSV)	-	
Spectrofluorimetric method (terbium sensitized)	4.92 × 10 <sup>-6</sup> -2.95 × 10 <sup>-5</sup> mol l <sup>-1</sup>	58.4 ng ml <sup>-1</sup> (by DPCAdSV)	-	pharmaceutical formulation	(13)
		3.88 × 10 <sup>-7</sup> mol l <sup>-1</sup>	1.29 × 10 <sup>-6</sup> mol l <sup>-1</sup>	standard samples	
		Proposed method	8.8 × 10 <sup>-8</sup> -8.8 × 10 <sup>-7</sup> mol l <sup>-1</sup>	2.8 × 10 <sup>-8</sup> mol l <sup>-1</sup>	
	1.1 × 10 <sup>-7</sup> -8.8 × 10 <sup>-7</sup> mol l <sup>-1</sup>	3.9 × 10 <sup>-8</sup> mol l <sup>-1</sup>	-	pharmaceuticals and spiked serum samples	



intensity was stable 60 min after all the reagents had been added. This is due to the rapid complex formation between  $Tb^{3+}$ , Dano and Cfx.

Investigating the effect of temperature, it was found that the fluorescence of the probe in the presence of analyte was constant at temperatures between 15–25°C. The quenched fluorescence intensity was increased at higher temperatures due to increase in the kinetic of molecules and the number of collisions (23). However, the fluorescence spectra were less repeatable at higher temperatures, and considering this, there was no significant improvement in the fluorescence intensity at 25°C and 35°C. Therefore, the room temperature was preferred which was more convenient and reliable as some spectrofluorimetry instruments are not equipped with a thermostat system. In the interference studies it was found that these compounds had negligible effect on the determination of  $1.1 \times 10^{-6}$  mol/l Cfx under optimal permission of 5 % error. Moreover, precipitation of serum samples using acetonitrile along with diluting the serum samples 100 times could eliminate the possible interference of proteins and other coexisting substances in the fluorescence intensities of the probe.

For clinical investigations such as pharmacokinetic studies, development of sensitive and selective analytical methods for the determination of drugs in biological fluids is required. Several methods (Table 5) have been reported for the quantitative determination of Cfx including spectrophotometry (4), spectrofluorimetry (13), High performance liquid chromatography (HPLC) (7) and voltammetry (9) methods. Most of these methods were time-consuming, tedious, and dedicated to sophisticated and expensive analytical instruments. Spectrophotometric methods are the most convenient techniques because of their inherent simplicity, high sensitivity, low cost, and wide availability in quality control laboratories. Unfortunately, the spectrophotometric methods reported for determination of cephalosporins in their pharmaceutical formulations were associated with some major disadvantages such as the lack of selectivity, tedious extraction procedures and a long process time. HPLC is the recommended method for the analysis of cephalosporins in pharmaceutical preparations and has its practical limitations, specially for routine analysis in a laboratory. Therefore, the development of new alternative spectrofluorimetric method for the determination of cephalosporins that can overcome the disadvantages of the existing methods is essential. Few methods are available in the literature for fluorimetric determination of Cfx. These methods are either suffers from interferences from other compounds, require expensive reagents or are suffer from narrow range of calibration curve. The proposed

fluorescence probe has a high sensitivity and wide range for Cfx determination at ng levels. It is also inexpensive and the reagents are easily available and environmental friendly. The  $Tb^{3+}$ -Dano complex has good stability and solubility in water and does not require the addition of luminescence enhancers. In addition, the proposed method could be applied for analysis of Cfx in human serum samples.

## Conclusion

A new fluorimetric method for determination of Cfx is proposed in this study. Under optimal conditions, the fluorescence intensity of  $Tb^{3+}$ -Dano system can be quenched by Cfx which indicates a linear relation between the quenched fluorescence intensity and Cfx concentration. This method was applied for the determination of Cfx in pharmaceutical tablets and spiked human serum samples. The method has the advantages such as simplicity, fast processing, low detection limits, wide linear range, and stability with relatively no interference with coexisting substances.

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## Conflict of Interests

All authors declare that they have no conflicts of interest.

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