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Determination of some non –steroidal anti-inflammatory drugs through quenching the fluorescence of lanthanide Tris complex

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Abstract

A new, simple and sensitive spectrofluorimetric method is developed for the determination of Piroxicam (PX), Tenoxicam (TX) and Lornoxicam (LX) .The proposed method involves the formation of complex with terbium (Tb^{3+}) in the presence of Tris buffer. The quenching of terbium fluorescence due to complex formation is monitored at λ_{em} 540 nm after using λ_{ex} 237 nm. A linear relationship is found between the concentration of the studied drugs and the quenching of the fluorescence of terbium-Tris complex. The optimum reaction conditions are studied and optimized .The proposed method is applicable over the concentration range 1.509×10^{-6} – 1.509×10^{-5} M (0.5-5 µg mL^{-1}), 2.96×10^{-6} – 1.48×10^{-5} M (1-5 µg mL^{-1}**)** and 1.34×10^{-6} – 1.34×10^{-5} M (0.5-5 µg mL^{-1}) with mean percentage recovery 99.66 ± 3.3, 100.79 ± 4.9 and 99.7±1.1 for PX, TX and LX respectively**.**The Correlation Coefficient is 0.9983 (n=8), 0.9989 (n=5) and 0.9999 (n=6) with limit of detection 0.2, 0.25 and 0.2 µg mL^{-1} for PX , TX and LX respectively**.** The proposed method is applied successfully for the determination of the three drugs in their pharmaceutical formulations.The results are compared favorably with those of official or reference methods.

Keywords:

Piroxicam, Tenoxicam, Lornoxicam, Terbium and Spectrofluorimetry

1. Introduction

Piroxicam (4-hydroxy-2-methyl-N-2-pyridinyl-2H-1,2– benzothiazine-3-carboxamide 1,1- dioxide) , Tenoxicam (4-hydroxy-2-methyl-N-2-pyridinyl-2H-tieno [2,3-e]-1,2-thiazine-3-carboxamide 1,1-dioxide) and Lornoxicam (6-Chloro- 4-hydroxy-2-methyl-N-2-pyridinyl-2H-tieno [2,3-e]-1,2-thiazine-3-carboxamide 1,1-dioxide), are non-steroidal anti-inflammatory and analgesic agents[1]. They belong to the class of oxicams; they are indicated in rheumatoid arthritis, osteoarthritis, extrarticular inflammation and acute gout [2]. Different methods were described for the determination of PX and or TX in pharmaceutical formulations and biological fluids. These methods include electro analytical methods such as voltammetry [3-6] and potentiometry [7, 8], spectrophotometric methods [9-12] and spectrofluorimetric methods [13-15]. The reported methods for determination of PX and or TX involve thin layer chromatography [16], high performance liquid chromatography [17-21] and electrophoresis [22]. Few methods were reported for determination of LX by spectrophotometry [23] and high- performance liquid chromatography [24-26].

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Lanthanide ions (especially Tb^{3+} and Eu $^{3+}$) tend to form stable chelates with organic ligands. These lanthanide chelates are characterized by large stocks shift, narrow emission bands and long fluorescence life times. The reaction of lanthanide ions with some organic compounds was used to improve the fluorimetric detection of certain organic analytes. This method is known as lanthanide –sensitized luminescence [27] and it was used for fluorimetric determination of some fluoroquinolones [28-30], tetracycline [31], anthranilic acid derivatives [32], ciclopirox olamine [33] and DNA [34]. Some organic compounds can quench the back ground luminescence of these ions. The quenching effect is more important with the chloride than the nitrate salts of the lanthanides, because the probability of collisions leading to energy transfer is larger for the chloride salts [27]. This quenching phenomenon was reported for the fluorimetric determination of some cephalosporines [35]. This work utilizes the quenching effect of the studied compounds on the fluorescence of terbium (Tb^{3+}) in Tris buffer pH 8.5 for the determination of these compounds in bulk as well as in some of their pharmaceutical formulations.

2. Experimental

2.1. Apparatus

BIO-TEK Model SFM 25 single beam spectrofluoremeter equipped with double monochromator system with reference beam compensation of source changes using 1cm quartz cell. Sensitivity adjusted to be 700 mV. Digital pH meter (Consort P-300) was used for adjustment of pH

2.2. Materials and Method

2.2.1. Reference samples

PX, TX and LX pure samples, kindly supplied by (EL-Obour Modern Pharmaceutical Industries Co, Egypt).The % purity of PX and TX was checked according to B.P[36] and was found to be (99.7) and (99.1) respectively. The purity of LX was provided by the company to be 99.6% and used as supplied.

2.2.2. Market samples

Felden tablet (Pfizer Co., Egypt) each tablet contains 20 mg of PX. Feldoral capsule, Feldoral suppository and Feldoral ampoule (Sedico Co., Egypt) are labeled to contain 20 mg of PX per capsule , suppository or ampoule . Tenocam capsule (Kahira Co., Egypt) each capsule contains 20 mg of TX. Xefo tablet (Manufactured by October Pharma S.A.E.6 October city for Egyptian Promotor center for Pharmaceuticals S.A.E. under liscence of Nycomid, Austaria) each tablet contains 8 mg of LX.

2.2.3. Reagents

All chemicals used are of analytical grade and are used without further purification**.** Terbium (III) Chloride hexahydrate 99.9٪ (Aldrich, Germany), 2×10^{-3} M methanolic solution was prepared. Tris buffer [Tris (hydroxyl methyl) amino methane] was obtained from (Nice Chemicals, India), 0.1 mol L^{-1} methanolic solution was prepared [36]. Methanol HPLC grade 99.9 % was obtained from (Sigma, Germany). Sodium hydroxide pellets was obtained from (Winlab, Leicestershire, U.K.). Sodium chloride was obtained from (Winlab, Leicestershire, U.K.). Hydrochloric acid HPLC grade 37 % was obtained from (Rediel –De-Haen, Germany).

2.3. Preparation of Sample Solutions

PX, TX and LX stock sample solutions (0.8 mg mL^{-1}) were prepared by dissolving 20 mg of each drug in 2 ml 0.2 mol L^{-1} NaOH in 25 mL volumetric flask and completed to volume with methanol. A working sample solution (40 µg mL^{-1}) of each drug was freshly

prepared by appropriate dilution of the stock solution with methanol. The stock solutions were kept in a refrigerator at approximately 4^0C and remained stable for at least 1 month [14].

2.4. Construction of Calibration Graphs

1.0 mL of Tris buffer pH 8.5 ± 0.2 was transferred in a series of 10 mL volumetric flasks followed by1.0 mL of terbium solution (2×10^{-3}M). Aliquots of the studied drugs working solutions equivalent to 5-50 μg for PX and LX and 20-50 μg for TX were added. The solutions were mixed well and allowed to stand for 15 minutes then they were completed to volume with methanol. The relative fluorescence intensity was measured at λ_{540} nm after excitation at λ_{237} nm. The final concentration of the studied drugs was plotted versus the calculated fluorescence quenching (F^0/F) to obtain the standard calibration graphs. Alternatively the corresponding regression equations (Stern- Volmer equation) [37] were derived.

2.5. Note

F^0: fluorescence of 0.2 m M solution of Tb^{3+} in Tris buffer and methanol without drug F: fluorescence of 0.2 m M solution of Tb^{3+} in Tris buffer and methanol after addition of the drug

2.6. Analysis of Dosage Forms

2.6.1. Felden and Xefo tablet:

Ten tablets were weighed and reduced to a fine powder .An amount equivalent to 20 mg of either PX or LX was accurately weighed and transferred to 25 mL volumetric flask, dissolved in 2 mL 0.2 mol L^{-1} NaOH and made up to volume with methanol. The solution was sonicated for 15 minutes, filtered with rejection of the first few milliliters. The produced solution (0.8 mg mL^{-1}) of the studied drug was used to proceed as under preparation of sample solution.

2.6.2. Feldoral and Tenocam capsules:

The contents of not less than ten capsules were transferred to a suitable tared container and mixed well. An accurately weighed amount equivalent to 20 mg of PX or TX was transferred to 25 mL volumetric flask, dissolved in 2 mL 0.2 mol L^{-1} NaOH and completed to volume with methanol. The solution was sonicated for 15 minutes, filtered with rejection of the first few milliliters .The produced solution is claimed to contain 0.8 mg mL^{-1}. The steps described under preparation of sample solution were followed.

2.6.3. Feldoral suppositories

Ten suppositories were weighed, cut into small pieces and transferred to a small porcelain dish. They were melted by stirring in a water bath to homogenize and then cooled. A portion equivalent to 20 mg PX was weighed into a beaker, melted and dissolved in 2 mL 0.2 mol L^{-1} NaOH and methanol by stirring at 60 ^{0}C for 5 minutes. The solution was cooled, filtered and diluted to 25 mL with methanol to produce stock solution containing 0.8 mg mL^{-1} PX. The produced solution was used to proceed as under preparation of sample solution.

2.6.4. Feldoral ampoules

The contents of ten ampoules were mixed and a volume equivalent to 20 mg PX was transferred to 25 mL volumetric flask, mixed with 2 mL 0.2 mol L^{-1} NaOH and completed to volume with methanol to obtain stock solution containing 0.8 mg mL^{-1} PX. The steps described under preparation of sample solution were followed.

Suitable aliquots of the above prepared solutions of different pharmaceutical preparations were treated as described under construction of calibration graphs. The results were calculated for each preparation from the corresponding regression equation.

3. Results and Discussion

3.1. Fluorescence spectral characteristics

The solution of terbium chloride in Tris buffer and methanol shows intense fluorescence**,** when excited at λ_{237} nm, two emissions bands at λ_{484} and λ_{540} nm are observed as shown in Fig.1.These bands are characteristic of terbium due to $^5D_4$-$^7F_6$ and $^7F_5$ transitions respectively [38].The fluorescence spectra of TX [5µg mL^{-1}] in methanol are represented in Fig.2.Weak signals are obtained at λ_{469}, λ_{651}, λ_{667} nm after excitation at λ_{237} , λ_{327} , λ_{337} nm respectively. Fig.1 and 2 show that, there is no interference from the fluorescence of the studied drugs on the emission peak of Tb^{3+}–Tris complex at λ_{540} nm, so it is the wavelength chosen for fluorescence measurement. At λ_{484} nm the fluorescence of the studied drugs interferes with that of Tb^{3+}–Tris complex, resulting in non-quantitative quenching effect. Reviewing the literature revealed that the weak native fluorescence of the studied drugs has no useful analytical application [14], Therefore the complexation reaction between terbium and the studied drugs is carried out to improve the fluorimetric determination of these drugs. Quantitative measurements are made by excitation at λ_{237} nm and measuring the fluorescence at λ_{540} nm where the studied drugs show no interference. When the studied drugs are added to Tb^{3+} they react with terbium forming stable complexes with no fluorescent properties which cause quenching of the fluorescence intensity of methanolic Tb^{3+}–Tris complex. This quenching is proportional to the concentration of the added drug. The quenching of the fluorescence signal of Tb^{3+} in Tris buffer after complexation with LX and PX are shown in Fig. 3 and 4, respectively.

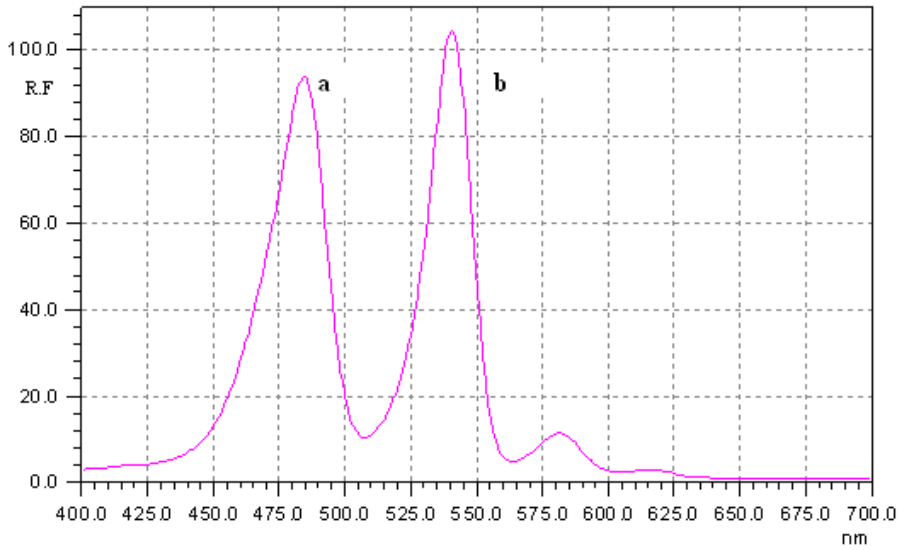


Fig 1. The emission spectra of terbium-Tris complex at [λ_{484}(a) and λ_{540} (b) after excitation at λ_{237}]

N.B. concentration of $TbCl_3$ is (0.2 mM)

R.F. = the relative fluorescence

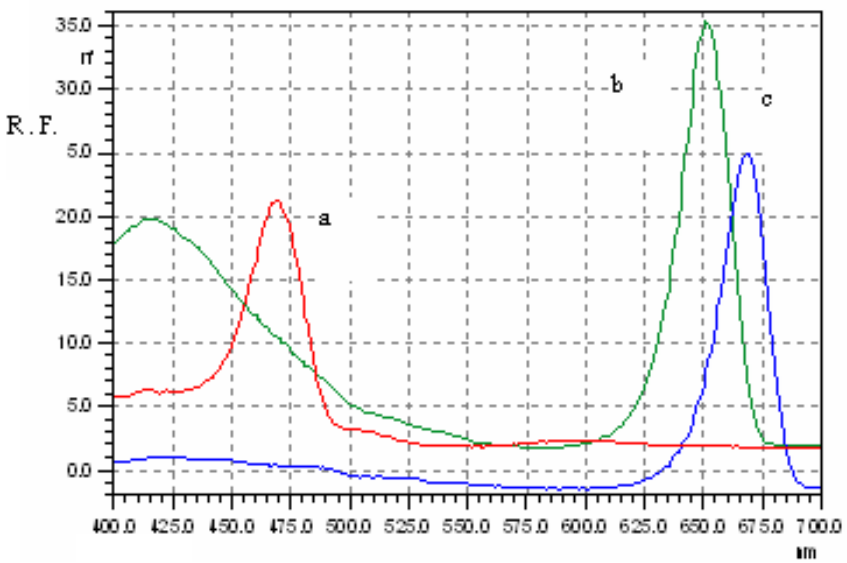


Fig 2. The emission spectra of TX (5µg mL-1) in methanol at (a) λ= 469, (b) λ= 651 and (c) λ= 667 nm after excitation at λ= 237 , λ= 327 and λ= 335, respectively

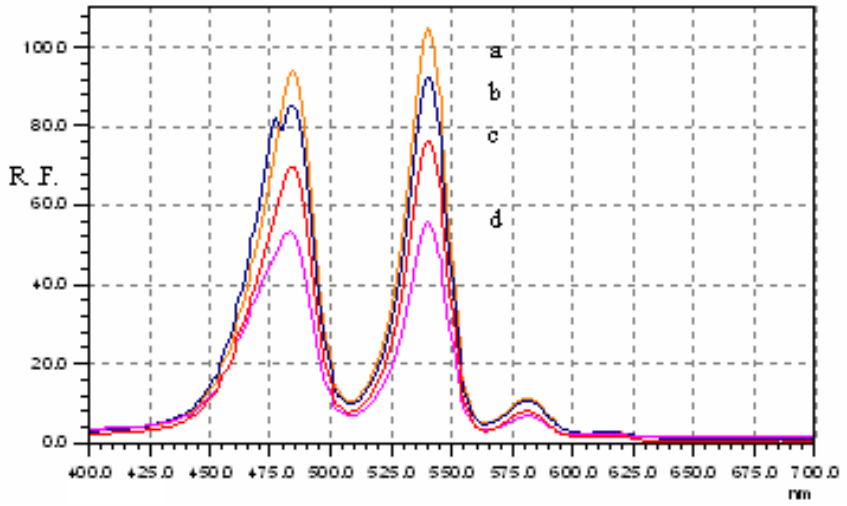


Fig 3. Stern Volmer plot of 1-{0.2mM $TbCl_3$ in tris buffer and methanol (a)) 2-(0.2mM $TbCl_3$ in tris buffer and methanol + [0.5 (b), 2.0 (c) and 5.0 (d) µg mL^{-1} of LX])

N.B. concentration of $TbCl_3$ is (0.2mM)

R.F. = the relative fluorescence

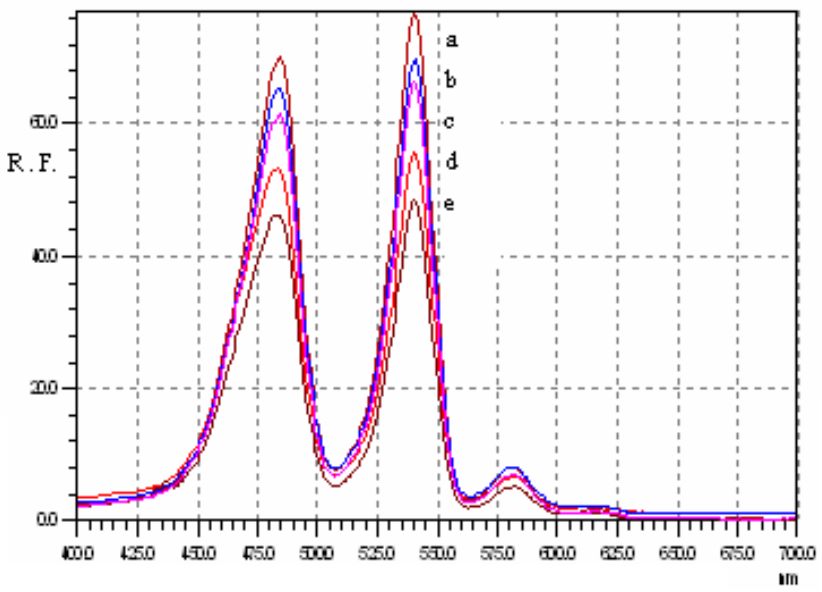


Fig 4. Stern Volmer plot of 1-{0.2mM $TbCl_3$ in tris buffer and methanol (a)) 2-(0.2mM $TbCl_3$ in tris buffer and methanol + [1.0 (b), 1.5 (c), 3.0 (d) and 5.0 (e) µg mL^{-1} of PX])

N.B. concentration of $TbCl_3$ is (0.2mM)

R.F. = the relative fluorescence

3.2. Optimization of the experimental conditions

Different experimental parameters affecting the relative fluorescence of Tb^{3+}–Tris complex and complex formation between the studied compounds and terbium were carefully studied and optimized. Such factors were changed individually, while others were kept constant. These factors include Volume of terbium chloride solution, Effect of pH and Effect of time on complex formation.

3.2.1. Volume of terbium chloride solution

The fluorescence of Tb^{3+} in Tris buffer and methanol at λ_{em} 540 nm (λ_{ex} 237 nm) was measured with increasing concentration of terbium and was found to increase quantitatively with terbium concentration as shown in Fig. (5). It was found that 1 mL of (2×10^{-3} mol L^{-1}) of Tb^{3+} is appropriate for reasonable fluorescence intensity.

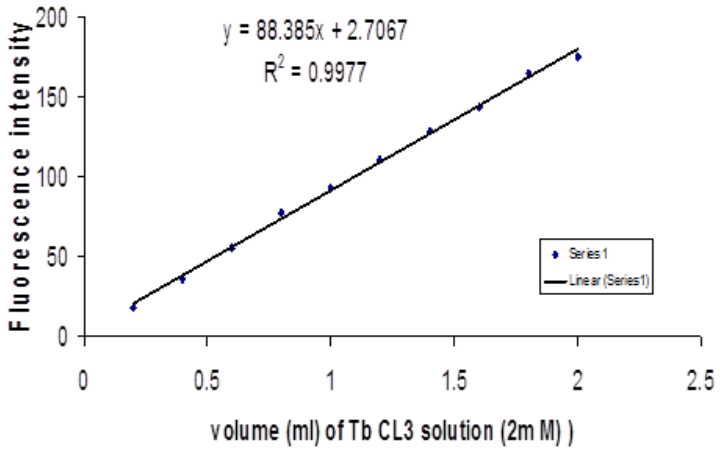


Fig 5. Calibration graph of the fluorescence of terbium chloride-Tris complex in methanol

3.2.2. Effect of pH

The influence of pH on relative fluorescence of Tb^{3+} and complex formation between terbium and the studied compounds was studied using different types of buffers covering wide range of pH. The results are represented in Fig. (6) and can be summarized as follow.

- *Acetate buffer*: the reaction was studied in acetate buffers of pH 4.7 and 5.5 where the fluorescence of terbium before and after addition of the drug was nil.
- *Borate buffer*: Solution of Tb^{3+} in borate buffer at different pH values (7.0, 8.0, 9.0and 10.0) ±0.2 showed intense fluorescence at λ_{540} nm. Quenching of this fluorescence upon addition of the studied drug was observed and was found to increase with increasing drug concentration but slight turbidity was observed which may be attributed to formation of terbium (III) hydroxide and caused measurements to be none reproducible.
- *Tris buffer*: The quenching of terbium fluorescence after addition of TX (3 µg mL^{-1} final concentration) was measured in different solutions of Tris buffer pH (8.0, 9.0, and 10.0) ± 0.2. The study revealed no significant difference in fluorescence quenching with change of pH. Tris buffer of pH 8.5 ± 0.2 was used for calibration.

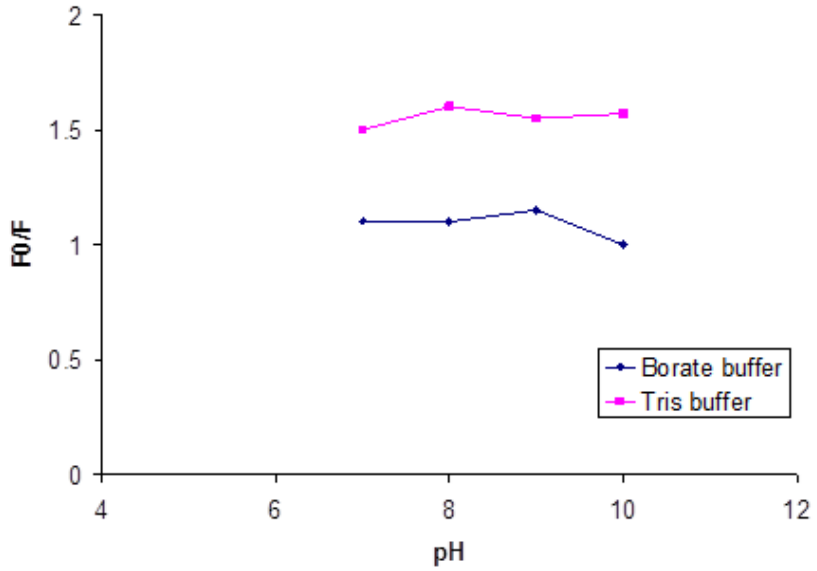


Fig 6. Effect of pH on complex formation between Terbium and the studied drugs (TX 3 µg mL^{-1} final concentration)

3.2.3. Effect of time on complex formation

The fluorescence of terbium–Tris solution after addition of TX (2 µg mL^{-1} final Concentration) was monitored after several time intervals. It was found that the complex was immediately formed and remained stable for at least 2 hours as shown in Fig 7. The fluorescence allover this study was measured after 15 minutes at room temperature.

3.2.4. Analytical features

After optimizing the conditions, the calibration graphs of PX , TX and LX were constructed by plotting the calculated fluorescence quenching of terbium solution (F^0/F) versus final concentration in µg mL^{-1}.The fluorescence –concentration plots were linear over the concentration range 1.509×10^{-6} −1.509×10^{-5} M (0.5-5 µg mL^{-1}), 2.96×10^{-6} −1.48×10^{-5} M (1-5 µg mL^{-1}) and 1.34×10^{-6} −1.34×10^{-5} M (0.5-5 µg mL^{-1}) with mean percentage recoveries

99.66 ± 3.4 (n=8), 100.79 ± 4.9 (n=5) and 99.70 ± 1.1 (n=6) for PX , TX and LX respectively. Analysis of the data gave the following regression equations:

$F^0/F = 0.1285\ C + 1.0128$ (r = 0.9983) for PX

$F^0/F = 0.2414\ C + 0.902$ (r = 0.9989) for TX

$F^0/F = 0.147\ C + 1.024$ (r = 0.9999) for LX

Where F^0 is the fluorescence of 0.2 m M solution of Tb^{3+} in Tris buffer and methanol without drug , F is the fluorescence of 0.2 m M solution of Tb^{3+} in Tris buffer and methanol after addition of the drug , C is the concentration of the studied drug in (μg mL^{-1}) and (r) is the correlation coefficient.

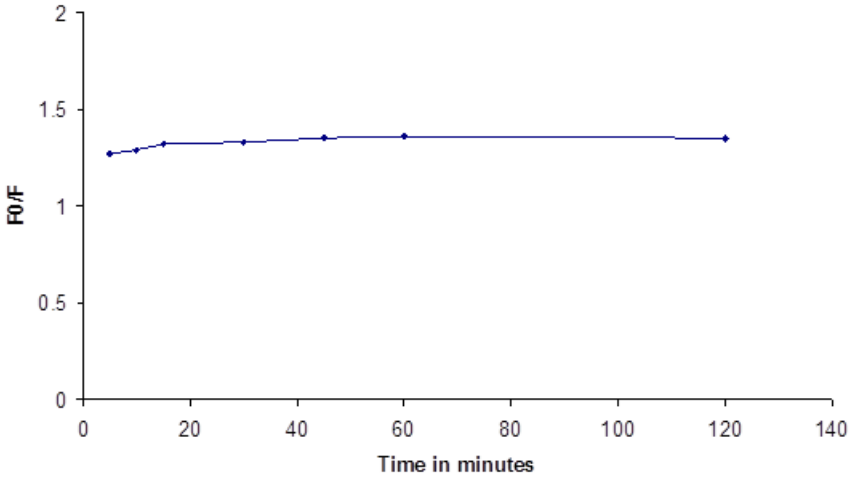


Fig 7. Effect of time on complex formation between Terbium and the studied drugs (TX 2 μg mL^{-1} final concentration) in Tris buffer

The limit of quantification (LOQ) was determined by establishing the lowest concentration that can be measured according to ICH Q2(R1) recommendations [39], below which the calibration graph is non linear(based on visual evaluation), while the limit of detection (LOD) was determined by evaluating the lowest concentration of the analyte that can be readily detected. The results of LOD and LOQ of the studied drugs by the proposed method are abridged in Table 1.

The proposed method was evaluated by studying the accuracy as percent relative error and precision as percent relative standard deviation. The results are abridged in Table 1. Statistical analysis [41] of the results obtained by the proposed method and the official method for PX [40] and the reference methods for TX [17] and LX [25] using Student's t-test and variance ratio F-test, shows no significant difference between the performance of our work and these methods regarding the accuracy and precision, respectively (Table 2).

3.3. Validation of the method

3.3.1. Linearity

The proposed method was tested for linearity, specificity, accuracy and precision. Linear regression equations were obtained. The regression plots showed linear dependence of fluorescence quenching of terbium solution on concentration of the studied drugs over the range cited in Table 1. The small values of the %RSD and %Er point out to the low scattering of the points around the calibration curve and high accuracy and precision of the proposed method.

Table 1. Performance data for the studied NSAI drugs by the proposed fluorescence quenching method.

Parameter	NSAI drugs		
	PX	TX	LX
Working range	0.5–5.0 μgmL^{-1}	1.0–5.0 μg mL^{-1}	0.5–5.0 μg mL^{-1}
Mean % Recovery	99.66	100.79	99.7 0
± S. D.	3.30	4.93	1.10
Relative standard deviation (%RSD)	3.30	4.90	1.10
Regression equation	F^0/F = 0.1285 C +1.0128	F^0/F = 0.2414 C + 0.902	F^0/F = 0.147 C + 1.024
Correlation coefficient (r)	0.9983	0.9989	0.9999
Standard deviation of the residuals ($S_{y/x}$)	0.0123	0.0201	0.0029
Percentage error %Er	1.17	2.18	0.45
Limit of Quantitation (LOQ)	0.5 μg mL^{-1}	1.0 μg mL^{-1}	0.5 μg mL^{-1}
Limit of Detection (LOD)	0.2 μg mL^{-1}	0.25 μg mL^{-1}	0.2 μg mL^{-1}

F^0: fluorescence of 0.2 m M solution of Tb^{3+} in Tris buffer and methanol without drug
F: fluorescence of 0.2 m M solution of Tb^{3+} in Tris buffer and methanol after addition of the drug
C = Concentration in μg mL^{-1}
LOQ and LOD are measured Based on Visual Evaluation

Table 2. Statistical analysis of the results obtained for the studied drugs in pure form by the proposed spectrofluorimetric method and comparison methods

Studied drug	Proposed method			comparison method
	concentration taken(μgmL^{-1})	concentration found(μgmL^{-1})	Recovery	% Recovery
PX	0.5	0.499	99.2	99.00
	1.0	0.995	99.5	98.00
	1.5	1.410	94.00	97.50
	2.0	1.970	98.50	95.30
	2.5	2.620	104.80	101.20
	3.0	2.990	99.67	103.60
	4.0	4.130	103.25	
	5.0	4.880	97.60	
Mean (X)± S. D.	99.66 ± 3.32			99.1 ± 2.9
Variance	11.02			8.41
Variance ratio F-test	1.31 (4.88) **			
Student's t-test	0.33 (1.78)**			
TX	1.0	1.09	108.90	95.60
	2.0	1.92	96.10	100.90
	3.0	2.93	99.76	103.20
	4.0	4.01	100.20	97.30
	5.0	5.05	101.00	102.00
Mean (X)± S. D.	100.79 ± 4.93			99.8 ± 3.2
Variance	24.3			10.24
Variance ratio (F-test)	2.37 (6.39) **			

Table 2. contiued

Studied drug	Proposed method			comparison method
Student's t-test	0.38 (1.85)**			
LX	0.5	0.49	98.00	97.50
	1.0	0.99	99.00	98.50
	2.0	2.01	100.5	96.60
	3.0	3.03	101.00	96.90
	4.0	3.98	99.5	101.00
	5.0	5.01	100.2	
_Mean(X) ± S. D.	99.7 ± 1.1		98.1 ± 1.78	
Variance	1.21		3.17	
Variance ratio (F-test)	2.62 (5.19) **			
Student's t-test	1.82 (1.83) **			

** The value in brackets are the tabulated ones at P =0.05

3.3.2. Accuracy and precision

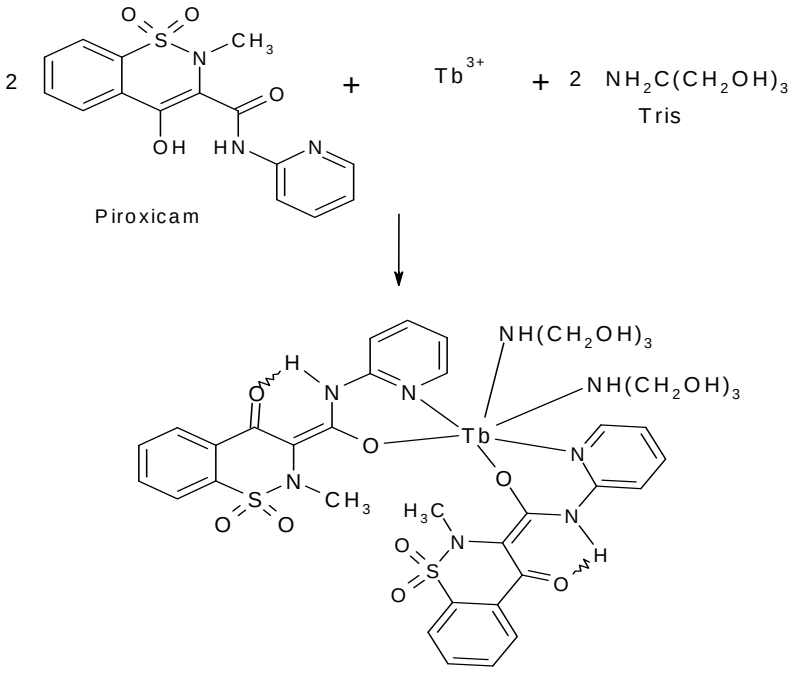
The results of the inter-day and intra-day accuracy and precision of the method have been summarized in Table 3. The inter-day and intra-day precisions were evaluated through replicate analysis of the studied drugs in pure form using different concentrations (2.0, 3.0, 4.0 μg mL^{-1}) and each concentration was measured three times a day for three consecutive days. The precision of the proposed method was fairly high, as indicated by the low values of SD and %RSD, respectively. Also the inter-day and intra-day accuracy was proved by the low values of %Er.

Table 3. Precision data for the studied drugs by the proposed fluorescence quenching method.

Parameter	Intra-day precision (Repeatability)			Inter-day precision (intermediate precision)		
concentration taken (μgmL^{-1})	2.0	3.0	4.0	2.0	3.0	4.0
Recovery of PX, %	100.40	101.80	99.3	98.50	99.80	102.80
	97.10	99.80	97.1	102.70	105.30	103.40
	96.70	105.10	98.4	95.50	101.90	100.40
Mean ($\overline{X}$)	98.10	102.20	98.40	98.90	102.30	102.20
± SD	2.10	2.70	1.80	3.60	2.80	1.60
%RSD	2.10	2.60	1.80	3.70	2.70	1.60
%Er	1.20	1.50	1.10	2.10	1.60	0.90
%Recovery of TX	96.50	97.50	102.4	94.1	97.40	100.90
	97.20	96.90	105.2	97.60	100.30	95.20
	94.80	99.60	100.00	101.20	94.50	103.90
Mean ($\overline{X}$)	96.20	98.00	102.50	97.60	97.40	100.00
± SD	1.20	1.36	2.60	3.60	3.10	4.40
%RSD	1.30	1.41	2.50	3.70	3.20	4.40
%Er	0.70	0.80	1.50	2.10	1.80	2.50
Recovery of LX, %	103.50	100.30	99.3	103.6	100.7	99.50
	99.10	98.30	96.00	100.2	104.2	96.00
	96.70	102.90	100.10	101.3	102.3	95.20
Mean ($\overline{X}$)	99.80	100.50	98.50	101.7	102.4	96.90
± SD	3.50	2.30	2.20	1.70	1.80	2.30
%RSD	3.50	2.30	2.20	1.70	1.80	2.40
%Er	2.00	1.30	1.30	0.97	1.0	1.40

3.4. Proposed mechanism of the reaction

In lanthanide–sensitized luminescence the intense luminescence originates from an intra molecular energy transfer through the excited triplet state of the ligand to the emitting resonance level of the ion followed by radiative emission from the cation. The efficiency of the energy transfer depends on the matching between the triplet level of the organic compound and the resonance level of the ion. The energy of the triplet level should be close to but higher than, that of the resonance level of the ion. In some instances, when the organic compound has a triplet state level below the excited state level of the lanthanide ion the organic compound can quench the back ground luminescence of the ion [27] . Several studies were made on TX and PX as chelating agents with uranyl (Uo_2 II), Fe III, Co II, Ni II, Cu II and other metals [42-44]. These studies mentioned that TX and PX act as neutral bidentate ligand coordinated to the metal ion via the pyridine –N and carbonyl-O groups. The structure of the complex formed between the studied drugs and Tb^{3+} is proposed to be as follow.



3.5. Application

3.5.1. Analysis of pharmaceutical formulations

The proposed method was applied for determination of PX in tablets, capsules, suppositories and ampoules, TX in capsules and LX in tablets. The results are shown in Tables 4 and 5. The results of the proposed and reference methods were compared in accordance with the Student's t –test and variance ratio F-test [41]. There were no significant differences between the calculated and tabulated values at P =0.05, demonstrating that the proposed method is as accurate and precise as the respective official and reference methods.

4. Conclusion

The data of the results given by this proposed procedure are indicative for higher sensitivity and reasonable selectivity. Furthermore, these findings are favorably comparable to other methods.

Table 4. Statistical analysis of the results obtained for PX in some pharmaceutical preparations using the proposed fluorescence quenching method and official method

Parameter	Feldoral capsule		Felden tablet		Feldoral suppository		Feldoral ampoule	
	Proposed method	Official method	Proposed method	Official method	Proposed method	Official method	Proposed method	Official method
concentration taken μgmL^{-1}	2.0	40.0	2.0	40.0	2.0	40.0	2.0	40.0
Recovery of PX, %	95.70	94.0	100.30	106.3	105.10	102.60	105.90	103.30
	98.95	96.50	99.60	102.00	104.40	107.30	102.50	107.30
	100.30	95.30	96.30	100.3	102.20	104.95	103.90	107.00
Mean (X)	98.30	95.30	98.70	102.86	103.90	104.95	104.10	105.90
± S. D.	2.40	1.26	2.1	3.1	1.50	2.30	1.70	2.23
Variance	5.76	1.59	4.41	9.61	2.25	5.29	2.89	4.97
Variance ratio F-test	3.6		2.18		2.35		1.72	
Student's t-test	1.92		1.93		0.66		1.11	

N.B. Tabulated t and F values at P = 0.05 are t = 2.132 and F = 19.00

Table 5. Statistical analysis of the results obtained for TX and LX in some pharmaceutical preparations using the proposed fluorescence quenching method and comparison methods:

Parameter	Tenocam capsule (TX)		Xefo tablet (LX)	
	Proposed method	Comparison method [17]	Proposed method	Comparison method [25]
concentration taken μgmL^{-1}	3.0	7.50	3.0	1.0
Recovery, %	96.30	97.80	99.80	96.00
	101.90	95.30	98.50	97.50
	100.20	100.10	96.60	100.60
	99.40			
	97.95			
	101.10			
Mean (X)	99.47	97.70	98.30	98.03
± S. D.	2.00	2.40	1.60	2.35
Variance	4.00	5.76	2.56	5.29
Variance ration F-test	1.44	(5.79) **	2.10	(19.00) **
Student's t-test	1.09	(1.894) **	0.165	(2.132) **

N.B. ** The value in brackets are the tabulated ones at P = 0.05 N

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