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Fluorimetric determination of some antibiotics in raw material and dosage forms through ternary complex formation with terbium (Tb^{3+})

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Abstract

A highly sensitive and specific fluorimetric method was developed for the determination of cefazolin sodium I, cefoperazone sodium II, ceftriaxone sodium III, and cefixime IV. The proposed method involves the formation of ternary complex with Tb^{3+} in the presence of Tris buffer. The quenching of the terbium fluorescence due to the complex formation was quantitative for the four studied drugs. The effect of pH, concentration of Tris buffer and terbium were studied. The formation of the complex was highly dependent on the pH. The optimum pH was found to be pH 8 for cefazolin sodium I, ceftriaxone sodium III, cefixime IV and pH 10 for cefoperazone sodium II. The optimum concentration for Tb^{3+} was found 1 ml of 10^{-4} M solution and for Tris buffer 1 ml of the prepared solution. Under the described conditions, the proposed method was applicable over the concentration range 8.79×10^{-6}–7.91×10^{-5}, 9.7×10^{-6}–$4.49 \times 10^{-}5$, 6.10×10^{-6}–2.50×10^{-5}, and 4.92×10^{-6}–2.95×10^{-5} mol with mean percentage accuracy of 99.79 ± 0.24, 98.97 ± 1.25, 100.05 ± 0.79, and 100.15 ± 0.54 for I, II, III, and IV, respectively. The proposed method was applied successfully for the determination of studied drugs in bulk powder and in pharmaceutical formulations. The results obtained by applying the described method were statistically analyzed and compared with those obtained by applying the official method. The proposed method was used as stability indicating method for the determination of the studied drugs in the presence of their degradation products.

Keywords: Fluorimetric determination; Antibiotics; Ternary complex; Terbium, Tb^{+}; Pharmaceutical formulations

1. Introduction

Cefazolin sodium I, cefoperazone sodium II, ceftriaxone sodium III, and cefixime IV belong to an important class of valuable clinical antibiotics, the cephalosporins. They are referred to the B-lactam antibiotics which are among the oldest and most frequently prescribed of the naturally occurring antimicrobial agents. The cephalosporins are commonly used for the treatment of infections caused by gram negative bacteria [1]. Published methods for determining cefazolin include colorimetry [2] and HPLC [3–5]. Other methods such as

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Table 1
Assay parameters for the proposed method

Compound	λ exc./λ em.	Concentration range mol	Regression equation		Correlation coefficient (r)	LOD [29]	LOQ [29]
			a	b			
Cefazoline	267/545	8.79×10^{-6}–7.91×10^{-5}	0.211	1.02×10^{6}	0.9999	1.39×10^{-6}	4.63×10^{-6}
Cefoperazone	240/485	9.70×10^{-6}–4.49×10^{-5}	−0.124	1.72×10^{6}	1.01	1.00×10^{-6}	3.34×10^{-6}
Ceftriaxone	270/548	6.10×10^{-6}–2.5×10^{-5}	−0.360	3.14×10^{6}	0.9998	5.09×10^{-7}	1.69×10^{-6}
Cefixime	288/580	4.92×10^{-6}–2.95×10^{-5}	0.724	2.29×10^{6}	1.01	3.88×10^{-7}	1.29×10^{-6}

ΔIf = a + b C. a, Intercept; b, slope and c, concentration in mol.

derivative spectrophotometry [6,7], adsorptive stripping voltammetry [8], differential pulse polarography [9], colorimetry [10,11], single-sweep oscillopolarography [12] and HPLC [13–15] have been reported for the determination of cefoperazone. A variety of analytical procedures have been reported for the determination of ceftriaxone viz. derivative spectrophotometry [16,17], colorimetry [18], differential pulse polarography and cyclic voltametry [9,19], HPLC [20–23] and densitometry [24,25].

Few methods have been published for the determination of cefixime. It was determined in dosage forms and biological fluids by HPLC [26–28] and colorimetry [18].

The USP 24 gives HPLC method for all the studied drugs in bulk powder and dosage forms [29].

Flourescence spectra of lanthanide ions especially, where they are chelated with ligands constituted the basis of a technique in microanalysis of many organic compounds. The main advantage of lanthanide chelates in fluorescence spectrometry include large Stokes shifts, narrow emission bands and long fluorescence life times [30]. In all applications of lanthanide ions, the intense luminescence originates from an intramolecular energy transfer through the excited state of the ligand to the emitting level of the lanthanide ion. Conversely, energy transfer from an organic compound to a lanthanide ion can be used to improve the fluorimetric analysis of organic analytes.

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 background luminescence of the ion. The sensitizing or quenching effect is more important with the chloride than the nitrate salts of the lanthanide, because the probability of collisions leading to energy transfer is larger for the chloride salt [31].

Based on energy level considerations and luminescence quantum yields, Tb^{3+} and Eu^{3+} are the best lanthanide ions to be applied to the determination of organic compounds.

Several compounds have been determined by using Tb^{3+} such as salicylates [32], proteins [31] and nalidixic acid [33].

The terbium (Tb^{3+}) metal ion has been the lanthanide of choice in most applications of ternary-complex formation. Therefore, the objective of this paper was to study the formation of ternary-complex between Tb^{3+}–Tris buffer and the studied drugs in an attempt to develop a fluorimetric method for their determination.

The present paper describes a specific and highly sensitive method for the determination of studied drugs. The method was based on the reaction of these drugs with terbium (Tb^{3+}) in the presence of Tris buffer at pH 8 and 10. The ability of these drugs to form ternary complexes with lanthanides specially Tb^{3+} initiated the present study.

2. Experimental

2.1. Apparatus

1) SHIMADZU RF-540 Spectrofluorophotometer, using quartz cell (1 × 1 × 4.5 cm), slit width 2.5 nm.
2) Digital pH meter, PW 9409 Pye Unicum.

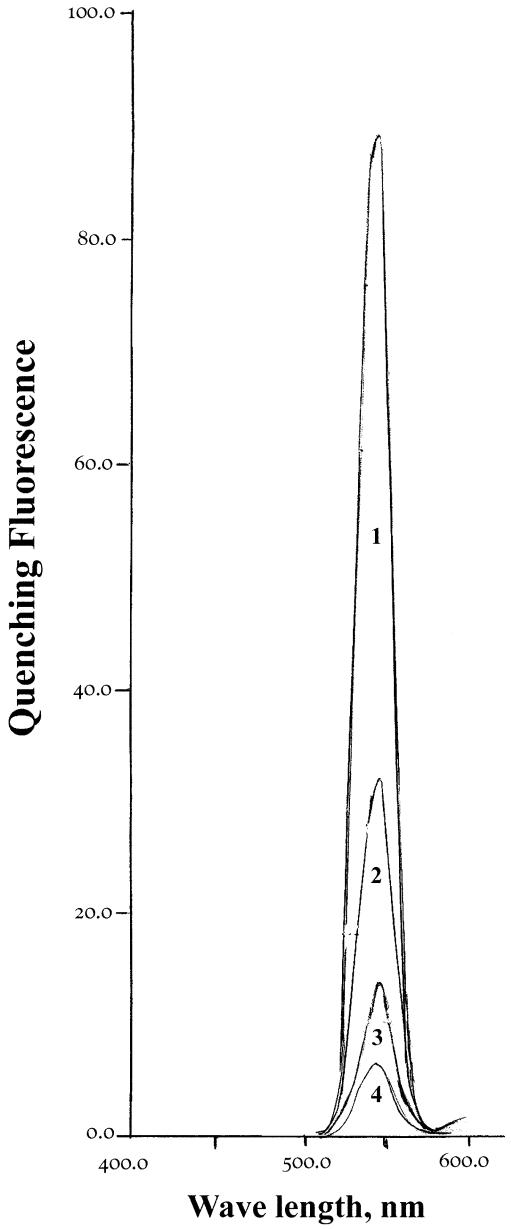


Fig. 1. Emission spectra of ceftriaxone at 548 nm. **1**, Tb^{+}-Tris buffer pH 8; **2**, Ceftriaxone (1.13×10^{-5} mol)-Tb^{3+}-Tris buffer ternary complex; **3**, Ceftriaxone-Tb^{3+}; **4**, Ceftriaxone (1.13×10^{-5} mol) in deionized water.

2.2. Materials and reagents

Cefazolin sodium, (Bristol Mayers Squipp, Cairo, Egypt), cefoperazone sodium, (Pfizer Co., Cairo, Egypt), Ceftriaxone sodium, (CID Co., Cairo, Egypt) and Cefixime, (Pharco, Cairo, Egypt). Their purities were certified and analyzed to be 100.12 ± 0.14, 99.75 ± 0.66, 99.83 ± 0.41 and 100.53 ± 0.75%, respectively, by the official HPLC method [29]. They were obtained as gifts from their companies and were used as working standards. Terbium oxide (Tb_2O_3) Sigma, 99% purity. A stock solution of Tb^{3+} (1×10^{-3} M) was prepared by dissolving the appropriate amount of terbium oxide Tb_4O_7 in the least amount of nitric acid and evaporating the solution till dryness, then the residue was dissolved in 50 ml of 2 M hydrochloric acid. A working solution (1×10^{-4}) was prepared by further dilution with distilled water. Tris buffer solution was prepared by dissolving 2 g of Tris and 2.4 g of sodium chloride in 100 ml water. Adjust the pH with 1 M sodium hydroxide or 1 M hydrochloric acid and then dilute to 200 ml with water. Sodium hydroxide, hydrochloric acid and methanol were analytical grade.

Table 2
Statistical comparison between the proposed method and official one

Compound	Recovery ± R.S.D.%	
	Proposed method	Official method [29]
Cefazoline	99.79 ± 0.24; F = 2.94 t = 1.85	100.21 ± 0.14
Cefoperazone	98.97 ± 1.25; F = 3.57 t = 0.24	99.75 ± 0.66
Ceftriaxone	100.05 ± 0.79; F = 3.71 t = 0.55	99.83 ± 0.41
Cefixime	100.15 ± 0.54; F = 1.93 t = 0.92	100.53 ± 0.75

Tabulated t-test and F-ratio at $P = 0.05$ and $n = 5$, 2.306 and 6.39, respectively.

2.3. Preparation of standard solutions

1) Cefazolin sodium, aqueous stock standard solution (1×10^{-3} M), freshly prepared.
2) Cefoperazone sodium, stock standard solution (1×10^{-3} M), freshly prepared by dissolving the appropriate amount in 25 ml volumetric

Table 3
Assay results for dosage forms of cefazolin, cefoperazone, ceftriaxone and cefixime using the proposed method compared statistically with the official method

Preparations	Found, mean ± R.S.D.%	Recovery ± R.S.D.%	Official method [29]
Totacef vial 1 g[a]	101.20 ± 0.95; F[j] = 2.68 t[i] = 1.94	99.67 ± 0.68	100.24 ± 0.58
Kefzole vial 0.5 g[b]	100.13 ± 0.48; F = 2.92 t = 1.25	99.54 ± 0.79	99.60 ± 0.82
Cefobide vial 0.5 g[c]	99.95 ± 0.77; F = 1.45 t = 0.39	100.33 ± 0.24	100.30 ± 0.64
Cefozone vial 1 g[d]	99.10 ± 1.58; F = 1.30 t = 2.25	99.56 ± 0.89	101.50 ± 1.80
Cefotrix vial 0.5 g[e]	99.97 ± 0.52; F = 3.06 t = 0.50	100.23 ± 0.41	99.56 ± 0.91
Cefotrix vial 0.250 g[f]	100.91 ± 0.84; F = 1.90 t = 0.52	101.21 ± 0.35	101.15 ± 0.61
Cefixime capsule 0.2 g[g]	98.64 ± 0.95; F = 1.34 t = 0.40	99.90 ± 0.16	98.90 ± 1.10
Cefixime capsule 0.4 g[h]	100.17 ± 0.87; F = 3.58 t = 0.98	99.15 ± 0.76	99.74 ± 0.46

[a] Each vial labeled to contain 1 g of cefazolin obtained from Bristol Mayers Squipp, batch no. M 73091.
[b] Each vial labeled to contain 0.5 g of cefazolin obtained from Lilly, Egypt, batch no. 780063.
[c] Each vial labeled to contain 0.5 g cefoperazone obtained from Pfizer Co, batch no. 72535209.
[d] Each vial labeled to contain 1 g Cefoperazone obtained from Eipico, Egypt, batch no. 975552.
[e,f] Each vial labeled to contain 0.5, 0.250 g Ceftriaxone obtained from T $_3$ A, Egypt, batch no. 010439 and 010443, respectively.
[g,h] Each capsule labeled to contain 0.2, 0.4 g cefixime obtained from Pharco, batch no. 754620, 534120, respectively.
[i] Tabulated t-value for $P = 0.05$ and eight degrees of freedom is 2.306.
[j] Tabulated F-ratio for $P = 0.05$ and F1 − F2 = 4 is 6.39.

flask in 3 ml methanol and complete to volume with water.

3) Ceftriaxone sodium, aqueous stock standard solution (1×10^{-4} M), freshly prepared.
4) Cefixime, stock standard solution (1×10^{-3} M), freshly prepared by dissolving the appropriate amount in 25 ml volumetric flask in 3 ml methanol and complete to volume with water.

3. Procedure

3.1. Calibration curve

Transfer 1.0 ml of terbium solution (1×10^{-4} M) for I, II and III and 2 ml for IV into a series of 10 ml volumetric flasks. Add aliquots of studied drugs each according to the working range as shown in Table 1, then add 1 ml of Tris buffer of pH 8 in case of I, III, IV and pH 10 in case of II. Complete to volume with distilled water. Measure the quenching fluorescent at 545, 485, 548 and 580 nm using 267, 240, 270 and 288 nm as an excitation wavelengths for I, II, III, and IV, respectively. Plot the concentration versus the % relative intensity (RI) to obtain the standard calibration graphs and compute the linear regression equations.

3.2. Procedure for dosage forms

3.2.1. For injection

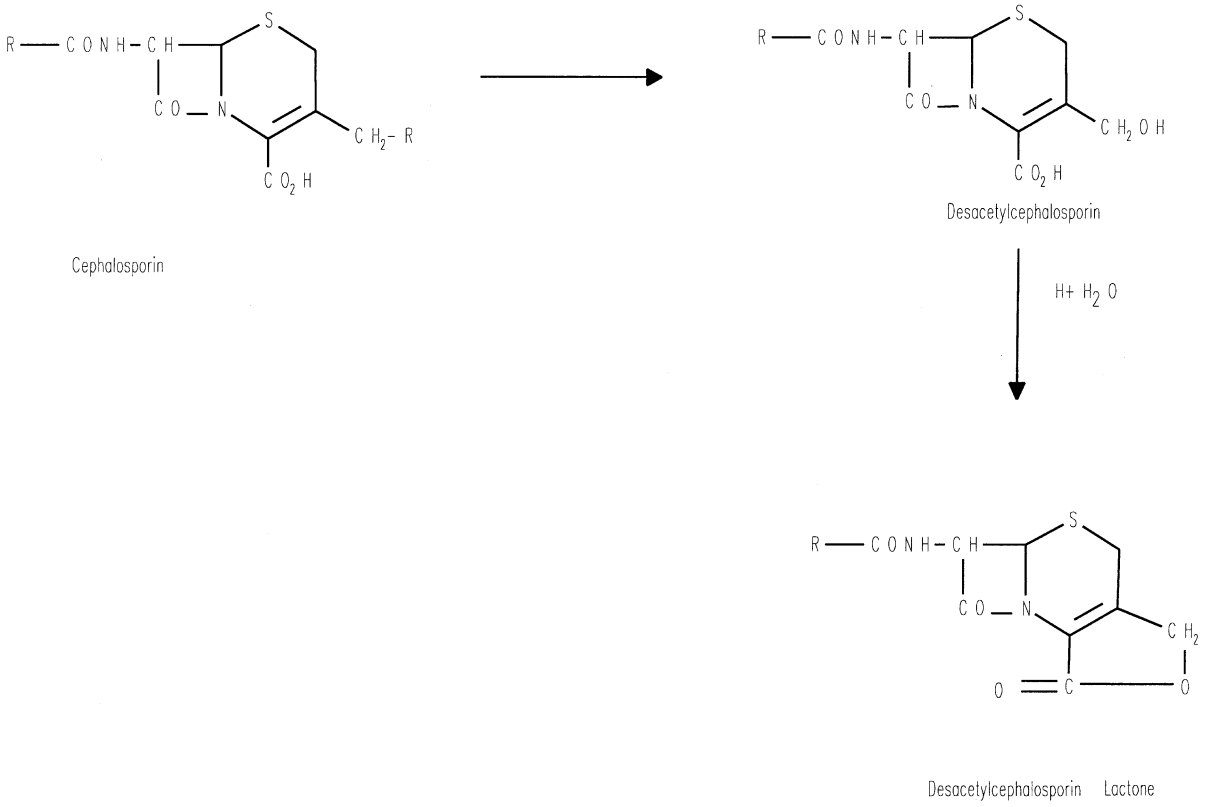
Constitute sterile powder in a volume of water accurately measured corresponding to the volume specified in the labeling. Dilute an accurately measured volume of injection quantitatively to obtain a solution of 1×10^{-3} M for I, II, IV and 1×10^{-4} for III. Carry out the procedure under calibration curve and calculate the concentration from the regression equation.

3.2.2. For capsules

Weigh and powder the contents of ten capsules. Accurately weigh an amount of the powder to obtain 25 ml of 1×10^{-3} M solution, dissolve in 3 ml methanol, complete to volume with water and filter. Proceed as described above.

4. Results and discussion

The solution of Tb^{3+} in Tris buffer have an intense fluorescence, if compared with its solution



Scheme 1.

in hydrochloric acid. On adding the drugs a fluorescence quenching was observed. The relative emission spectra of test and blank solutions are shown in Fig. 1. The decrease in relative fluorescence intensity was proportional to the concentration of the added drug.

The cefazolin I, cefoperazone II, ceftriaxone III and cefixime IV–Tb^{3+}–Tris buffer ternary complex were found to exhibit fluorescence quenching at wavelengths as shown in Table 1.

The optimum conditions for drugs–Tb–Tris ternary complex formation were studied. The fluorescence quenching of the drug solution was measured over a pH range 7–11 by using Tris buffer. The fluorescence quenching was observed at pH 8 for I, III, IV and pH 10 for II.

The effect of terbium concentration on the fluorescence intensity was studied by increasing the volume of terbium chloride (1×10^{-4} M). It was found that 1 ml for I, II and III and 2 ml for IV were appropriate for fluorescence intensity.

The ternary complex is formed immediately and the fluorescence signal remains stable for at least 4 h.

The fluorescence signal was linearly related to the concentration in the range 8.79×10^{-6}–7.91×10^{-5}, 9.7×10^{-6}–4.49×10^{-5}, 6.10×10^{-6}–2.50×10^{-5}, and 4.92×10^{-6}–2.95×10^{-5} mol with mean percentage accuracy of 99.79 ± 0.24, 98.97 ± 1.25, 100.05 ± 0.79, and 100.15 ± 0.54 for I, II, III, and IV, respectively. The regression equations were computed as shown in Table 1.

The suggested method was successfully applied to the determination of the studied drugs in both raw materials and pharmaceutical dosage forms. The precision was calculated by performing five analysis of each sample. The relative standard deviation (R.S.D.) was determined and the results

obtained were compared statistically with those obtained by applying the official methods as shown in Table 2.

The stoichiometry of the reaction was studied by the molar ratio and was found to be 1:3 (Tb^{3+}:drug).

The proposed method was applied for the determination of the studied drugs in their pharmaceutical dosage forms and the validity was assessed by applying the standard addition technique. The results obtained are presented in Table 3.

Tables 2 and 3 show that the calculated *t*-test and F ratios are less than the corresponding theoretical values indicating that there is no significant difference between the proposed method and the official ones with respect to accuracy, precision and repeatability.

Furthermore, trials to use the proposed method as stability-indicating method was successfully. The studied drugs were degraded using 0.02 M sulfuric acid in an oven at 105 °C for 4 h. The solution was cooled, neutralized with 0.02 M sodium hydroxide, concentrated to about 2 ml, transferred to 10 ml volumetric flask and diluted to volume with methanol. The procedure was completed as in reference [34] for the separation of degradation products.

The proposed method was applied on the degradation product, no quenching was observed.

The Tb^{3+} complex formation necessitate the presence of carboxylic group. Upon the drug degradate, decarboxylation and opening of the B-lactam may occurs as shown in Scheme 1.This leads to in failure of complex formation with terbium and there is no quenching occurs.

Accordingly, the proposed method was applied on a mixture containing 20% degradation product. The results obtained determined only the intact drug. Thus, the proposed method can be used as stability-indicating method for the studied drugs.

5. Conclusion

The proposed method is characterized by its simplicity compared with the official method. Also, the proposed method is selective, rapid, cheap and need neither expensive solvents nor sophisticated apparatus. It can be used as stability-indicating, so it determines the intact drugs in the presence of their degradation products. The described spectrofluorimetric method could be applied successfully in quality control laboratories.

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