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Spectrophotometric Methods for the Determination of Ceftiofur and Tulathromycin in Bulk and Dosage Forms

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Authors' contributions

This work was carried out in collaboration among all authors. Author AMA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors NSR and FAF managed the analyses of the study. Author AMA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Two simple, accurate and precise spectrophotometric methods were developed for the determination of two veterinary drugs, ceftiofur and tulathromycin in pure form as well as in pharmaceutical formulations. The first one (Method A) based on the reducing action of ceftiofur on Fe (III) to Fe (II) in its complex with 1, 10- phenanthroline (ferrin complex) to give the orange-red colored ferroin complex that exhibits an absorption maximum at 511 nm. Ceftiofur exhibited good linearity in the concentration range of 0.3-3.0 µg mL^{-1}.The second method **(**Method B) depended on formation of a binary complex between tulathromycin and eosin Y in in the presence of carboxy methylcellulose as surfactant. Under the optimum conditions, the binary complex showed absorption maxima at 556 nm. The method obeyed Beer's law over concentration range of 1.0–15.0 µg mL^{-1}. The proposed methods were used for determination of the studied drugs in pharmaceutical formulation; maxfur® powder and draxxin® injections with mean recoveries of 99.57and 99.71%, respectively. The validity of the methods was further proved by applying the standard addition technique. A proposal of the reactions pathways were described.

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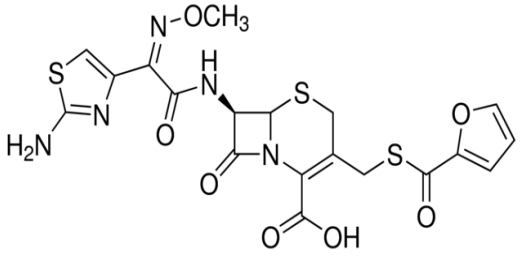
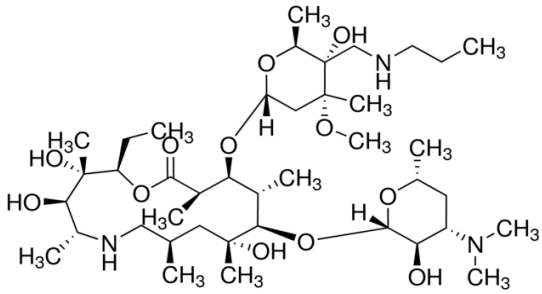
Keywords: Ceftiofur; tulathromycin; spectrophotometry; 1; 10- phenanthroline; eosin Y.

1. INTRODUCTION

Ceftiofur (CEFT) Fig. 1 is a semisynthetic, beta-lactamase-stable and third-generation cephalosporin. It binds to and inactivates penicillin-binding proteins located on the inner membrane of the bacterial cell. This results in the weakening of the bacterial cell wall and causes cell lysis [1]. CEFT is broad-spectrum antibiotic has been used for bovine and swine respiratory disease, foot rot and metritis infections in cattle [2]. A number of HPLC [3-5], LC/MS [6-8], capillary electrophoresis [9], UV-Vis spectrophotometric [10-13] and voltametric [14,15] methods were reported for the quantification of CEFT in dosage forms and or biological samples.



Ceftiofur



Tulathromycin

Fig. 1. Chemical structure of ceftiofur and tulathromycin

Tulathromycin (TULA) Fig. 1 is a novel long-acting semi-synthetic tribasic macrocyclic antimicrobial agent; therefore it has given the chemical subclass designation of the triamilide. Its actions are related to direct inhibition of essential protein biosynthesis by selective binding to bacterial 50S ribosomal subunits, so it approved for use in the treatment and prevention of bovine respiratory disease and the treatment of swine respiratory disease [16]. Few LC/MS [17-22] and electrochemical method [23] were reported for the determination of TULA in in pharmaceutical formulations and or different biological tissues. Compared with the reported methods the proposed methods have some distinct advantages; the reported spectrophotometric methods [10-13] were less sensitive. Although the reported HPLC and LC/MS methods [3-8,17-22] are sensitive enough, they are precluded due to higher cost and non- availability of such instruments in each laboratory in developing countries. No reported spectrophotometric methods were reported for determination of TULA in its dosage form.

So, there is still need for developing a simple and sensitive spectrophotometric methods for determination of CEFT and TULA and the suggested methods meets their require-ments.

The aim of this work is to introduce simple, selective and economical spectro-photometric methods for the determination of the studied drugs in their pharmaceutical preparations.

2. MATERIALS AND METHODS

Pure CEFT sodium was kindly provided by Kahera Pharmaceutical & Chemicals Industrial Company, Cairo, Egypt. Its purity was found to be 99.98% as stated by the supplier. Pure TULA was purchased from Pfizer Company, Cairo, Egypt. Its purity was found to be 99.00% as stated by the supplier. Maxfur® sterile powder; (B.N.01608368) each 1 ml of reconstituted solution contains CEFT (as sodium salt) equivalent to 50 mg CEFT (a product of Kahera Pharmaceutical & Chemicals Industrial Company, Cairo, Egypt) purchased from commercial sources. Draxxin® solutions for injection; (B.N. B178907) each 1 mL contain 100 mg TULA (a product of Fareva Amboise, France, imported by Zoetis import Egypt) purchased from commercial sources. 1,10 Phenanthroline (Merck, Germany); 7.5 $x10^{-3}$ M of Phen-Fe (III) mixture [24] prepared by dissolving 0.1487 gm of O-phenanthroline monohydrate and 0.1189 gm ammonium ferric sulfate in 5 mL 1 N hydrochloric acid and then diluted to 100 mL with water. Ammonium ferric sulfate ($NH_4Fe\ (SO_4)_2.12H_2O$) was obtained from Winlab, U.K. Eosin Y(Merck, Darmstadt, Germany); $2x10^{-3}$ M aqueous

solutions were prepared by dissolving 0.14 gm in 100 mL distilled water [25]. Glacial acetic acid, sodium acetate anhydrous, hydrochloric acid and ethanol purchased from EL-Nasr Company, Egypt. Surfactants; carboxy methylcellulose (CMC), tween 80, citrimide, sodium dodecyle sulphate (SDS) were provided from El-Nasr Pharmaceutical Chemicals Company (ADWIC), Abu Zaabal, Egypt and were prepared as a 0.1% w/v solution . Methanol was purchased from TEDIA (USA) and acetonitrile was provided from Sigma-Aldrich (Germany). 0.2 M Acetate buffer was prepared by mixing various volumes of 0.2 M acetic acid and 0.2 M sodium acetate solutions to obtain the required pH value [26].

2.2 Instrumentation

Shimadzu (Kyoto, Japan) UV-1601 PC, UV-Visible double-beam spectrophotometer with matched 1 cm path-length quartz cells. Absorption spectra were recorded on a fast scan speed, setting slit width to be 1 nm and sampling interval to be auto. Hanna pH-meter (Romania) equipped with glass–calomel electrode combination. Ultrasonic bath: BHA-180 T (Abbotta, USA).

2.3 Methods

2.3.1 Preparation of stock solutions

- Stock solution of CEFT containing 1mg /mL was obtained by dissolving 25 mg of the sample in 25 mL methanol. The stock solution was further diluted with methanol to get standard working solutions 20 µg/mL.
- Stock solution of TULA containing 0.1 mg /mL was obtained by dissolving 10 mg of the sample in 100 mL methanol.

2.3.2 Linearity

Method (A): Accurately measured aliquots of the stock solution equivalent to (3.0-30 µg /mL) of CEFT were transferred into in a series of 20 mL test tubes. Then 2 mL of 7.5 $x10^{-3}$ M Phen-Fe (III) and 1.1 mL of 0.2 M acetate buffer (pH 4.4) were added and mixed well. The tubes were heated in water bath at 90°C for 30 min then cooled, transferred quantitatively to 10 mL volumetric flasks and completed to the mark with distilled water. The absorbance at 511 nm was recorded against a similarly prepared reagent blank. The measured absorbance values were plotted verse the final concentration in µg /mL to get the calibration curve and the regression equation was derived.

Method (B): Accurately measured aliquots of the stock solution equivalent to (10-150 µg /mL) of TULA were transferred into a series of 10 mL volumetric flasks. 1.5 mL of 0.1% CMC and 1.6 mL of $2x10^{-3}$ M Eosin Y solution were added to each flask. The solutions were mixed well before the addition of 2.5 mL of 0.2 M acetate buffer (pH 4.3) to each flask. The flasks were completed with distilled water to the mark and the absorbance was measured at 556 nm against reagent blank. The measured absorbance values were plotted against the final concentration in µg /mL to get the calibration curve and the regression equation was obtained.

2.3.3 Application to pharmaceutical formulations

Method (A): Three sterile Maxfur® powder were accurately weighed and thoroughly mixed well. An accurately weighed amount of mixed powder equivalent to 25.0 mg was transferred to 25 mL volumetric flask and completed to volume with methanol to get a solution containing 1 mg/mL CEFT. The stock solution was further diluted with methanol to get standard solutions 20 µg/mL and was analyzed following the procedures described under "linearity". The concentration of the drug was calculated from the corresponding regression equation.

Method (B): Three draxxin injection solutions were mixed well and aliquot of the mixed solution equivalent to 100 mg was transferred to 100 mL volumetric flask and completed to volume with methanol to obtain stock solution claimed to contain 1 mg /mL. 2.5 ml from stock solution diluted to 25 ml with the same solvent to prepare standard working solution contained 0.1 mg /mL. TULA was analyzed following the procedures described under "linearity". The concentration of the drug was calculated from the corresponding regression equation.

3. RESULTS AND DISCUSSION

Method (A): The well-known reducing properties of B- lactam antibiotics which may be ascribed to their sulphar content [27] was used here as a basis for the reaction between CEFT and Phen-Fe (III) reagent. The spectrum of pure CEFT was presented in Fig. 2A, the maximum of

absorbance 291 nm. When CEFT reduce Fe (III) to Fe (II) in its complex with 1, 10-phenanthroline (Ferrin complex), it give orange-red colored Ferroin complex and exhibit a bathochromic shift at 511 nm. Fig. 2B. Thus the suggested reaction mechanism can be illustrated as follow in Scheme 1.

Method (B): TULA lacks the suitable chromophores for UV or fluorimetric detection. As TULA has three secondary amino groups; therefore it can form an ion pair red complex with eosin via electrostatic interaction between the most basic center in the drug molecule (amino groups) and the carboxylate anion of the dye. This primarily occurs in an acidic solution (pH 4.3), increasing the electron delocalization of eosin Y and producing a bathochromic shift of the dye (at 556 nm); Fig. 3. Thus the suggested reaction mechanism can be illustrated as follow in Scheme 2.

3.1 Optimization of the Reaction Conditions

Effect of pH and buffer volume: Studying the effect of 0.2 M acetate buffer revealed that 1.1 mL of pH 4.4 ± 0.1 and 1.5 mL of pH 4.3 ± 0.1 was found to be optimal for ferroin complex and ion pair complex respectively; Fig. 4 (A-D).

Effect of Reagents Volume: It was observed that 2 ± 0.2 mL of Phen-Fe (III) mixture was the optimum volume for development of the color with CEFT; Fig. 5A. 1.6±0.1 mL of eosin Y reagent was found to be sufficient to give maximum absorbance with TULA; Fig. 5B.

Effect of Time and Temperature: For CEFT, maximum color intensity was increased by increasing the temperature to 85°C, remain stable to 100°C. So the reaction was carried out at 90°C for 25 min; Fig. 6 A,B. The intensity of the final color was stable for at least one hour.

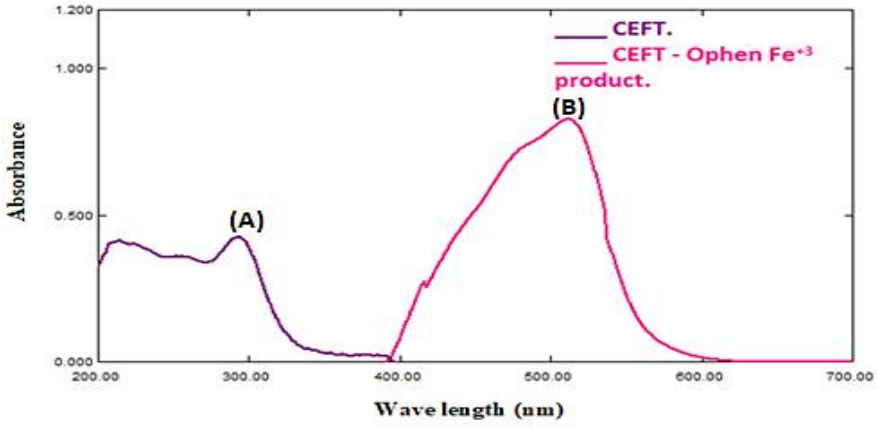
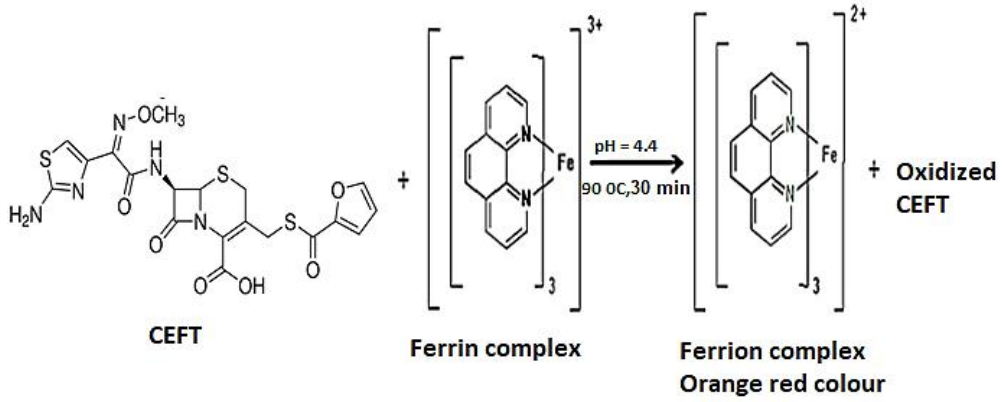


Fig. 2. Absorption spectra of: (A) CEFT in methanol (10 µg/ mL), (B) CEFT (2.4 µg /mL) phen-Fe (III) reaction product at pH 4.4



Scheme 1. Suggested reaction pathway of reduction of Phen-Fe (III)

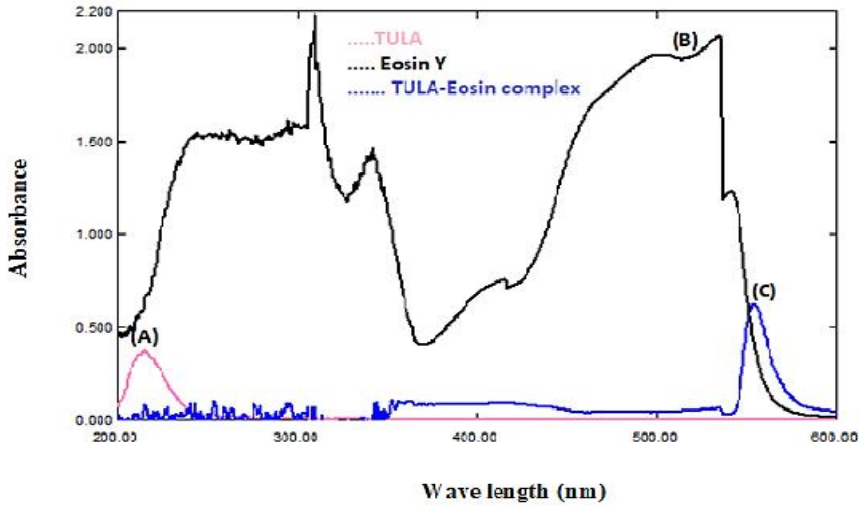
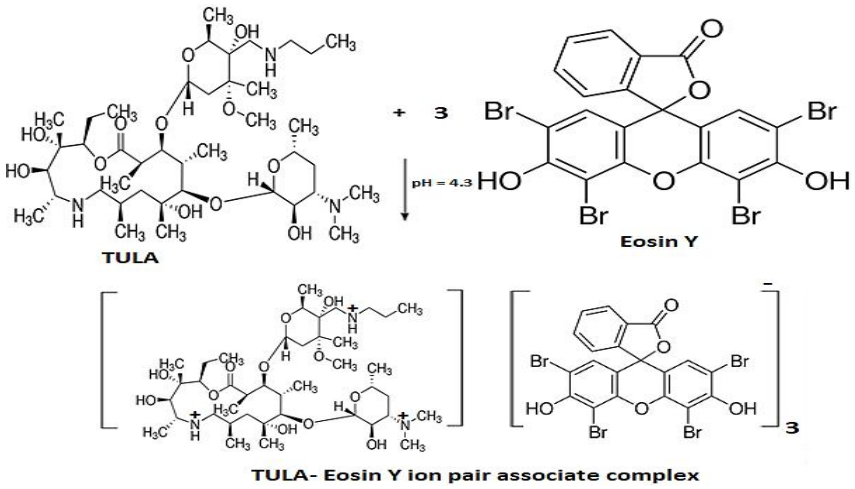


Fig. 3. Absorption spectra of: (A) TULA in methanol (1mg/ mL), (B) Blank eosin Y(2 × 10^{-3} M) in water, (C) Eosin Y binary complex with TULA (10 µg/mL) at pH 4.3



Scheme 2. Proposed mechanism for the reaction between TULA and eosin Y

Complete color development was produced instantaneously at room temperature between TULA and eosin Y and remained stable for further one hour. Increasing the temperature resulted in formation of a precipitate which may be due to coagulation of the formed complex.

Effect of type and volume of surfactant: Due to the slight solubility of complexes formed with eosin Y in aqueous acidic solutions, different nonionic surfactant such as CMC, tween 80, citrimide and SDS were tried to solubilize and stabilize the formed complex between TULA and eosin Y. CMC gave the highest absorbance and reproducibility; Fig. 7A. 1.5 ± 0.5 mL of 0.1% CMC was found to be sufficient to give maximum color intensity; Fig. 7B.

Effect of diluting solvent: Water, methanol, absolute ethanol and acetonitrile were tried as diluting solvent for the reaction between CEFT and Phen-Fe (III) mixture .The highest sensitivity was developed by using water as diluting solvent; Fig. 8.

3.2 Stoichiometry of the Reactions

Job's ratio method [28,29,30] was applied for determination the stoichiometric ratio between the cited drugs and corresponding reagent. When using 7 × 10^{-3} M equimolar solution of CEFT and Phen-Fe (III) mixture, the ratio of 3:7

(drug: reagent) was observed; Fig. 9A. The reaction proceeds in the ratio of 1:3 for TULA to eosin Y using 2 × 10^{-3} M equimolar solution of the drug and the reagent; Fig. 9B.

Table 1. Regression parameters for the determination of CEFT and TULA by the proposed spectrophotometric method

Parameter	CEFT	TULA
$\lambda_{max\ (nm)}$	511	556
Linearity range (µg mL^{-1})	0.3-3.0	1.0-15.0
Slope ±S.D	0.3372 ±3.134 $x10^{-3}$	0.055±2.51$x10^{-4}$
Intercept±S.D	0.0291 ±6.034 $x10^{-3}$	0.065±2.175$x10^{-3}$
SD of residuals	7.755 $x10^{-3}$	3.26$x10^{-3}$
Correlation coefficient (r)	0.9998	0.9999
LOD (µg mL^{-1})	0.054	0.119
LOQ (µg mL^{-1})	0.179	0.395
Accuracy (R%)	100.33	100.15
Precision (RSD %)*		
Intraday [a]	0.75-1.72	0.36-1.10
Interday [b]	1.11-1.69	1.54-1.56
Standard addition Mean ± SD	100. 37±0.551	99.63±1.198

**Each result is the average of the three separate determinations. [a] within the day, [b] Three consecutive days.*

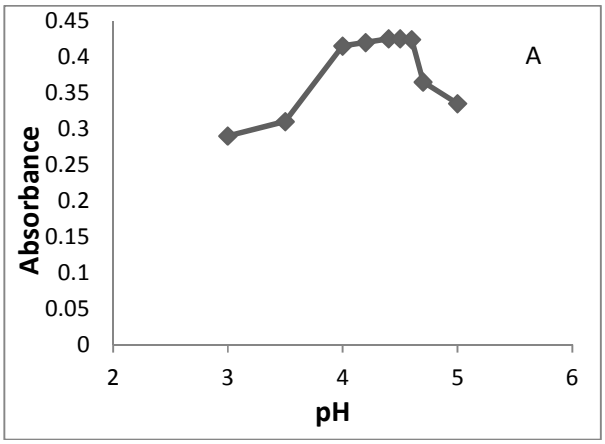


Fig. 4. (A) Effect of pH of 0.2 M acetate buffer on the absorbance value of 1.4 µg/mL for CEFT with Phen-Fe (III) mixture

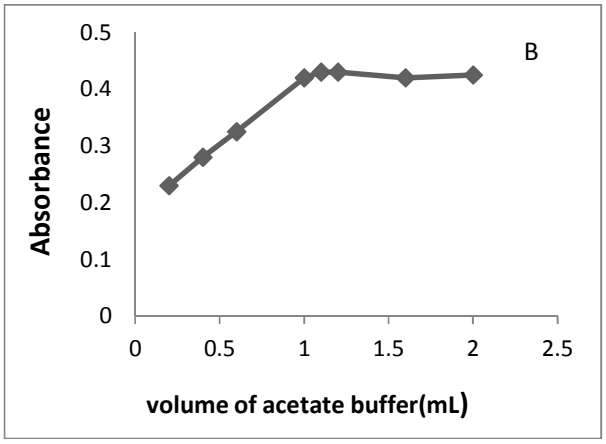


Fig. 4. (B) Effect of volume of 0.2 M acetate buffer on the absorbance value of 1.4 µg/mL for CEFTwith Phen-Fe (III) mixture

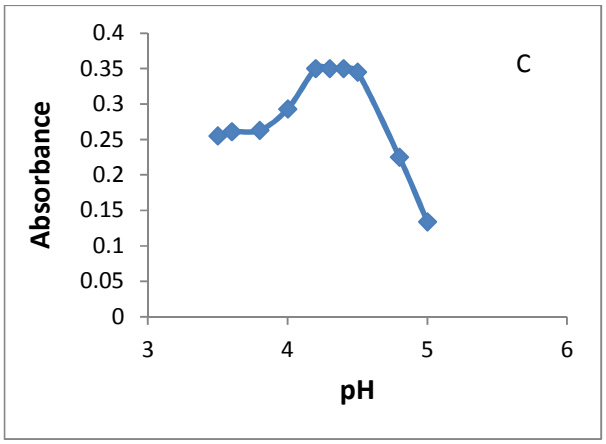


Fig. 4. (C) Effect of pH of 0.2 M acetate buffer on the absorbance value of 6 µg/mL for TULA with eosin Y (2×10^{-3} M)

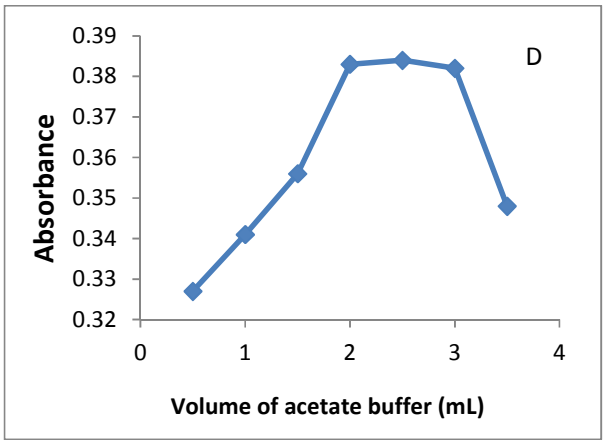


Fig. 4. (D) Effect of volume of 0.2 M acetate buffer solution on the absorbance value of 6 µg/mL for TULA with eosin Y (2×10^{-3} M)

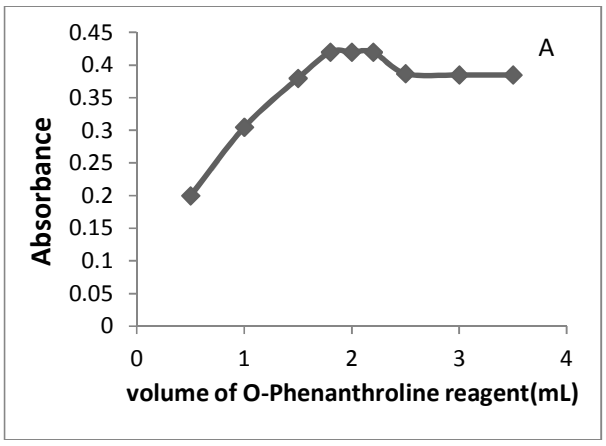


Fig. 5. (A) Effect of volume of Phen-Fe (III) mixture on the absorbance value of product with of 1.4 µg/mL for CEFT

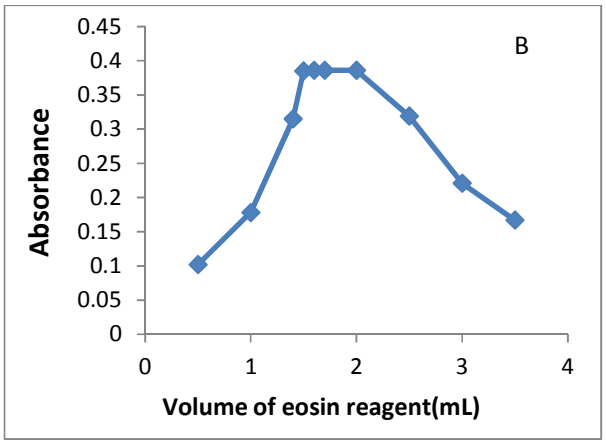


Fig. 5. (B) Effect of volume of eosin (2×10^{-3} M) on the absorbance value of product with 6 µg/mL for TULA

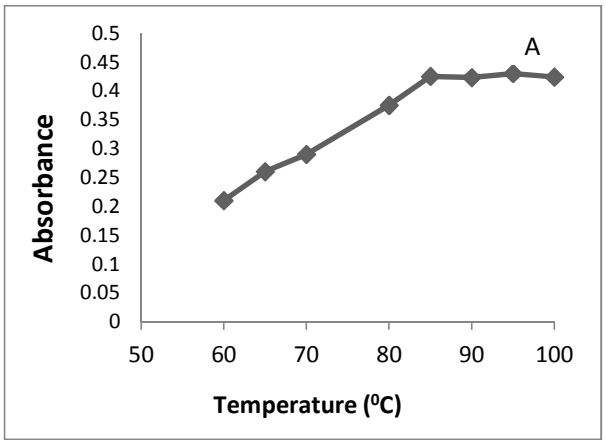


Fig. 6. (A) Effect of temperature on the absorbance value of product with of 1.4 µg/mL for CEFT

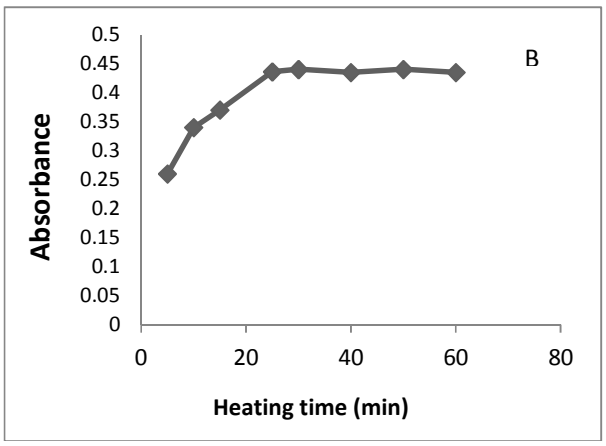


Fig. 6. (B) Effect of time on the absorbance value of product with of 1.4 µg/mL for CEFT

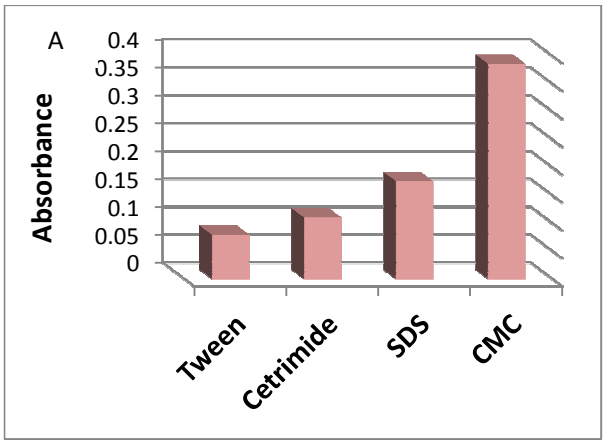


Fig. 7. (A) Effect of different types of surfactant on the absorbance of reaction products of 6.0 µg/mL TULA with Eosin Y (2×10^{-3} M)

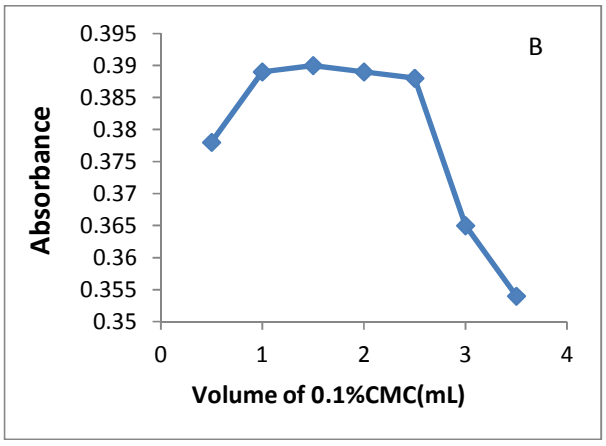


Fig. 7. (B) Effect of volume of 0.1% CMC on the absorbance of the reaction product of 6.0 µg/mL TULA with Eosin Y (2×10^{-3} M)

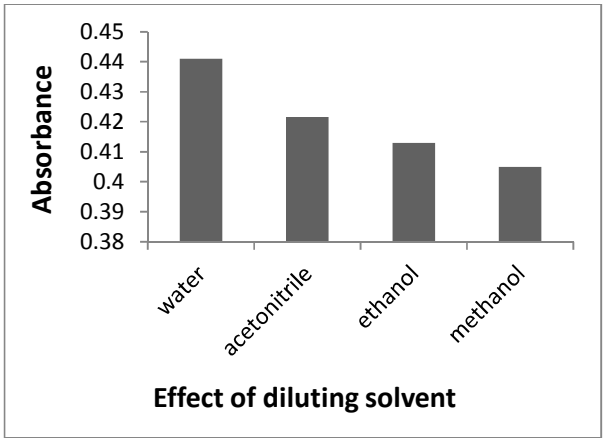


Fig. 8. Effect of diluting solvent on the absorbance value of product with of 1.4 µg/mL for CEFT

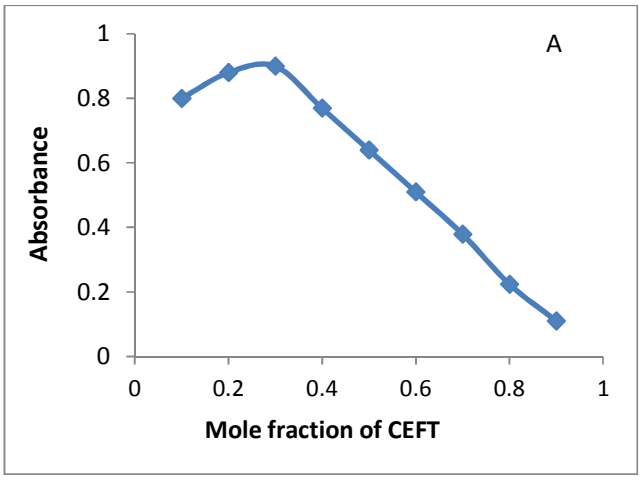


Fig. 9. (A) Stoichiometry of the reaction of CEFT with Phen-Fe (III) reagent (7×10^{-3} M) by Job's method

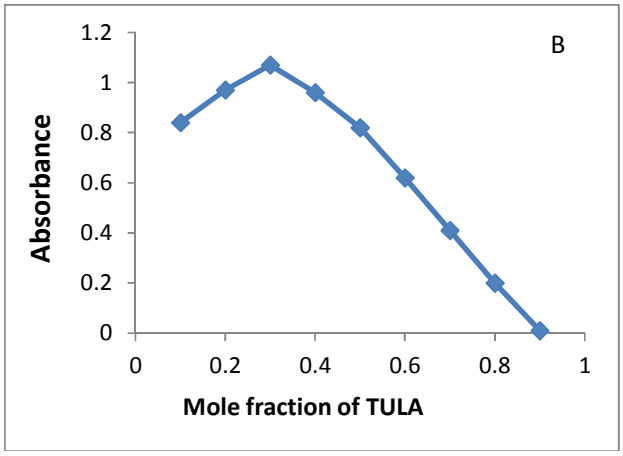


Fig. 9. (B) Stoichiometry of the reaction of TULA with eosin (2×10^{-3} M) by Job's method

3.3 Method Validation

The validity of the proposed methods were tested according to ICH Q2 (R1) recommendations [31].

1. Linearity- Under the described experimental conditions, linear calibration curves between absorbance to respective drug concentration were obtained through the concentration ranges of 0.3-3.0 µg/ mL for CEFT and 1.0-15.0 µg/ mL for TULA. The correction coefficients were 0.9998 and 0.9999 respectively indicating good linearities; Table 1.

2. Accuracy and precision- Evaluation of accuracy and precision of the proposed methods were performed by analyzing three CEFT or TULA concentration levels in triplicates on the same day or on three different days. Accuracies calculated as R% which ranged from 98.9% -101.5 % or 99.41% -101.04% with the two drugs, respectively. Intraday precision (RSD %) ranged from 0.75% to 1.72% and 0.36% to 1.10%, while inter day precision ranged from 1.11% to 1.69% and 1.54% to 1.56% for both drugs respectively indicating good repeatability and reproducibility of the suggested the methods; Table 1.

3. Selectivity- The selectivity of the method was investigated by observing any interference encountered from the common excipients of TULA and CEFT dosage forms. It was found that these compounds did not interfere with the results of the proposed method as shown in Table 2.

Table 2. Determination of CEFT and TULA in their pharmaceutical dosage forms

Parameters	Proposed method				Reported spectrophotometric method [13]		
	Taken (μg mL^{-1})	Found* (μg mL^{-1})	% Recovery	%RSD	Taken (μg mL^{-1})	Found (μg mL^{-1})	% Recovery
	0.6	0.599	99.9	1.04	5	4.9543	99.09
CEFT	1.5	1.48	99.1	1.49	10	10.091	100.91
Maxfur® powder B.N.01608368	3	2.99	99.7	1.14	15	14.954	99.69
Mean %± S.D.			99.57±0.42				99.89±0.93
t-test		0.567(2.776)					
F-test		4.95 (19.00)					
	8	8.15	101.56	0.48			
TULA	10	9.90	98.83	0.77			
Draxxin® injection (B.N. B178907)	15	14.84	98.73	0.67			
Mean %± S.D.			99.71±1.61				

The values between parentheses are the tabulated t and F values at P = 0.05 [32]

-The reported method of CEFT depended on UV spectrophotometric determination at 292 nm using methanol as blank [13]

3.4 Applications to Pharmaceutical Formulations

The suggested methods were successfully applied for the analysis of CEFT and TULA in maxfur® powder or draxxin® injections. The mean recoveries ± SD were 99.57±0.42 for CEFT and 99.71**±**1.61 for TULA; Table 2. For CEFT; results obtained for the determination of the drug in maxfur® powder was statistically compared to those obtained by the reported method [13] revealing no significant difference [32]; Table 2.

To prove the accuracy of the proposed spectrophotometric methods, the standard addition technique was applied. The results of the assay of CEFT or TULA in pure form by the proposed method were compared with results of recovery of pure in standard addition technique. The mean recoveries of pure added were calculated to be 100.37± 0.551 for CEFT and 99.63±1.19 for TULA; Table 1.

4. CONCLUSION

Simple, sensitive and accurate visible spectophotometric methods were developed for the determination of CEFT and TULA in pure powdered form and their pharmaceutical formulations. For first time; TULA can be determined spectrophotometrically using Eosin Y as ion-pairing agent. Spectrophotometric method is still extensively used in research laboratories and hospitals due to low cost, simplicity, portability and ease of operation as it not required tedious extraction procedures. This makes the methods suitable for routine analysis in quality control laboratories.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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