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Spectrophotometric method for determination of Cefixime in its pure form and in pharmaceutical preparations using diazotization reaction and coupling with Chromotropic acid reagent

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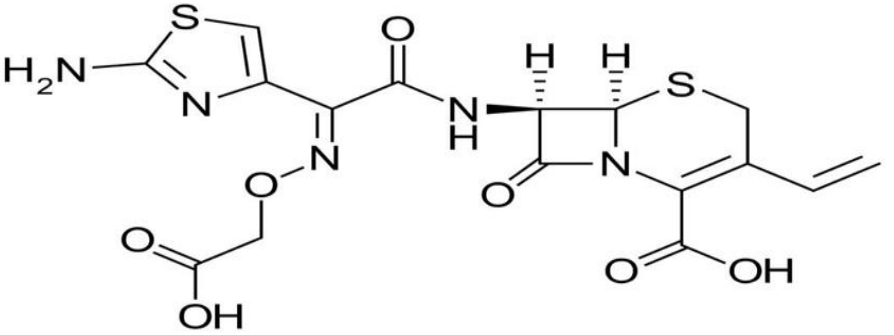
***Abstract*---**A new study includes development of a swift, sensitive and Cheap spectrophotometric method to estimation of Cefixime as pure and in its dosage forms. In this method cefixime was displaced with an equivalent amount of sodium nitrite solution in acidic medium to form resultant diazonium salt that will be coupling with Chromotropic acid reagent in basic medium to yield an red azo dye that has maximum absorption at 514 nm versus its blank solution. Under optimum conditions, the concentration range obeyed Beer's law is 0.5-20 µg/ml and the excellent determination coefficient was (R^2= 0.9991) and molar absorptivity 2.36×10^4 l/mol.cm. .The detection limit (LOD) and quantification limit (LOQ) were found to be 0.322 and 0.748 µg/ml, respectively. A relative error% was calculated and found in the range -2.75% to 4.02% , while the precision (RSD) was estimated as ≤ 1.36% .The stoichiometry of the resulting azo dye was found to be 1:1 Cefixime : Chromotropic acid . The proposed method was successfully applied to assay of Cefixime in tablets .

***Keywords*---**cefixime, diazotization, chromotropic acid, spectrophotometry.

Introduction

Cefixime (CFX): (6R,7R)-7-{[2-(2-Amino-1,3-thiazol-4-yl)-2-(carboxymethoxyimino)acetyl] amino}-3-ethenyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.



(CFX) ($C_{16}H_{15}N_5O_7S_2$) M.wt.= 507.5 g/mol

CFX is found in white crystals, melting point ranges from 220-250, slightly soluble in water, soluble in alcohol. CFX is considered one of the most important and active medicines of the third generation of cephalosporins (Troy and Beringer, 2005). Orally active cefixime has anti-pathogenic activity Such as anaerobic, Enterobacteriaceae, Gram-negative species such as Escherichia coli, Klebsiella, Haemophilus influenzae, including B-lactamase producing strains (Katzung, 2006; Sayed et al., 2013). Due to the low toxicity of CFX and its mild side effects, this drug was used to treat multidrug-resistant enteric fever and pharyngitis in children.(Attimarad *et al.*, 2013).

After searching in the previous literature, we found a large number of methods for the determination of cefixime using different techniques such as spectrophotometric methods (Naeem *et al.*,2015),(Nisreen *et al.*,2019), (Farha, 2013), HPLC (Zahra *et al.*, 2018), (Madhura *et al.*, 2011), (Madan *et al.*, 2015), Voltammetry (Rajeev *et al.*, 2011), fluorescence quenching (Muhammad, 2021). The suggested method gives great results for assessment CFX in pharmaceutical dosages .

Apparatus

For all absorbance measurements and absorption spectra used the double beam UV-VIS spectrophotometer (T92-PG INSTRUMENTS) with 1.0-cm glass cells was used. And a professional Benchtop pH meter BP3001.for the pH measurements .

Chemical substances

All chemicals used are of high purity and obtained from Fluka, BDH and Merk companies.
CFX stock solution (500 µg/ ml) : A 0.050 g of pure CFX was dissolved in about 5 ml of ethanol and the volume was then made up to 100 ml with distilled water in

a Volumetric flask and kept in a dark bottle. A working solution (100 µg /ml = 1.97×10^{-4} M) of CFX was prepared by diluting 20 ml of the stock solution with distilled water in a 100 ml calibrated flask.
Chromotropic acid (CRO-A) stock solution(1% m/v):A 1.0 g of pure CRO-A was dissolved in about 10 ml of distilled water With shaking until dissolved and then the volume was completed to 100 ml with distilled water in a volumetric flask and kept in a dark bottle.
Sodium nitrite stok solution (1.97×10^{-2} M): 0.136 g of Sodium nitrite was dissolved in a small amount of distilled water and completed the volume to 100 ml by same solvent using the calibrated flask. The working solution (1.97×10^{-4} M) was prepared by diluting 1 ml of the stock solution with distilled water in a 100 ml calibrated flask and then put this solution in a dark flask.
Hydrochloric acid solution (1M): Prepared by diluting 8.6 ml of the concentrated acid (Fulka) to a 100 ml with distilled water in calibrated flask.
Sodium hydroxide solution (1M): Prepared by dilution of the concentrated volumetric (BDH) solution(10 M) with distilled water to 1 L and then put it in a plastic bottle.

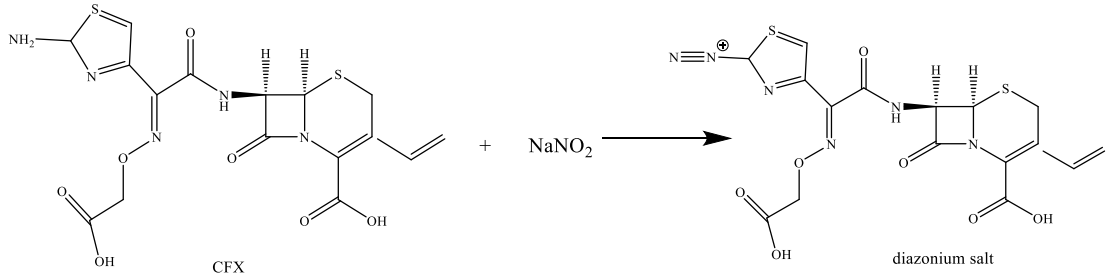
Tablet solution (winex) (100 µg CFX /ml): Contents of 5 tablets (each tablet contains 200 mg of CFX) were finely powdered, mixed thoroughly and weighed 0.01224 g that equivalent to 0.01 g of CFX and was dissolved in 10 ml distilled water with gentle heating and after filtration of the solution, the volume was completed to 100 ml by distilled water in a volumetric flask.

Tablet solution (suramix) (100 µg CFX /ml): Contents of 5 tablets (each tablet contains 400 mg of CFX) were finely powdered, mixed thoroughly and weighed 0.01236 g that equivalent to 0.01 g of CFX and was dissolved in 20 ml distilled water with gentle heating and after filtration of the solution, the volume was completed to 100 ml by distilled water in a volumetric flask.

Results and Discussion

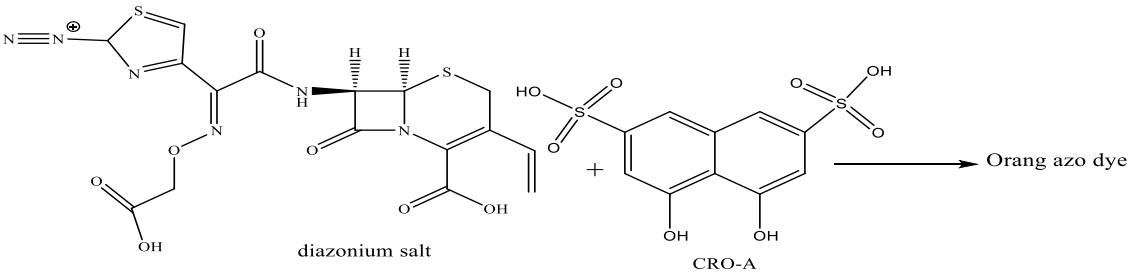
Preliminary conditions

Under the reaction conditions, CFX was reacted with equivalent amount of $NaNO_2$ in an acidic solution to obtained the corresponding diazonium salt.(Scheme 1).



Scheme 1

Then the diazonium salt was coupled with CRO-A in alkaline medium to form an intensely orange colored azo-dye.(Scheme 2).



Scheme 2

The orange azo dye displayed maximum absorption at 514 nm versus the blank solution. The dye intensity was found to be relative to the amount of CFX originally present in the solution.

The optimum conditions

Effect of acidic medium

The diazotization reaction need to acidic solution , therefore, we studied the effects of different amounts of different acid solutions at 1M concentration such as HCl, H_2SO_4, HCOOH, HNO_3, CH_3COOH on absorbance of azo dyes.The experimental results are shown in Fig. 1.

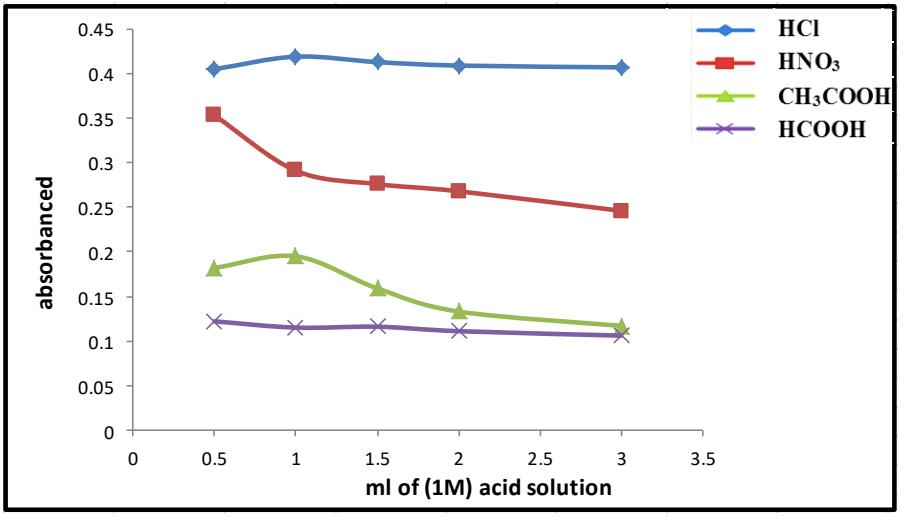


Fig. 1: Effect of acidic medium on absorbance of azo dye

The Results in Fig. 2 show that 1 ml of 1M HCl give maximum stability, sensitivity, and it is sufficient for complete the diazotization reaction.

The effect of the diazotzation time on absorbance of azo dye

In this study we measured the absorbance of azo dye after waiting for different times for completed the diazotzation reaction before add the reagent solution. The

data of figure 2 showed that the 5 minutes Sufficient to give maximum absorption.

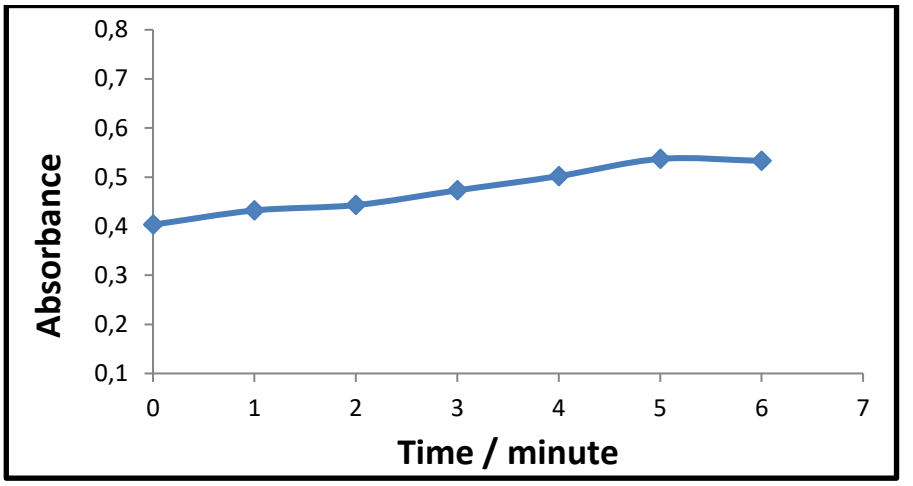


Fig. 2: Effect of time on the diazotization reaction

Effect of CRO-Ae reagent amount

The effect of different amounts (0.5-2.5) ml of 1% CRO-A reagent on the absorption of azo dyes was investigated. The results in table. 1 show that 2.0 ml 1% CRO-A is the optimum volume for giving high absorption and a reasonable coefficient of determination (R^2 = 0.9992) for the coupling reaction. So has been selected as coupling agent.

Table 1: Effect of amount of reagent

ml of 1 % reagent	Absorbance / ml of SFX					
	0.5	1	1.5	2.0	2.5	R^2
0.5	0.082	0.165	0.239	0.309	0.496	0.9435
1.0	0.128	0.303	0.391	0.569	0.778	0.9852
1.5	0.208	0.326	0.441	0.639	0.855	0.9908
2.0	0.295	0.539	0.812	1.079	1.308	0.9992
2.5	0.238	0.436	0.559	0.728	0.975	0.9925

The effect of coupling time

In this study the effect of the time for completing of coupling reaction between the diazotized CFX and CRO-A reagent, was studied by leaving the reagent solution at room temperature for different times after the addition of the CRO-A solution. (Fig. 3).

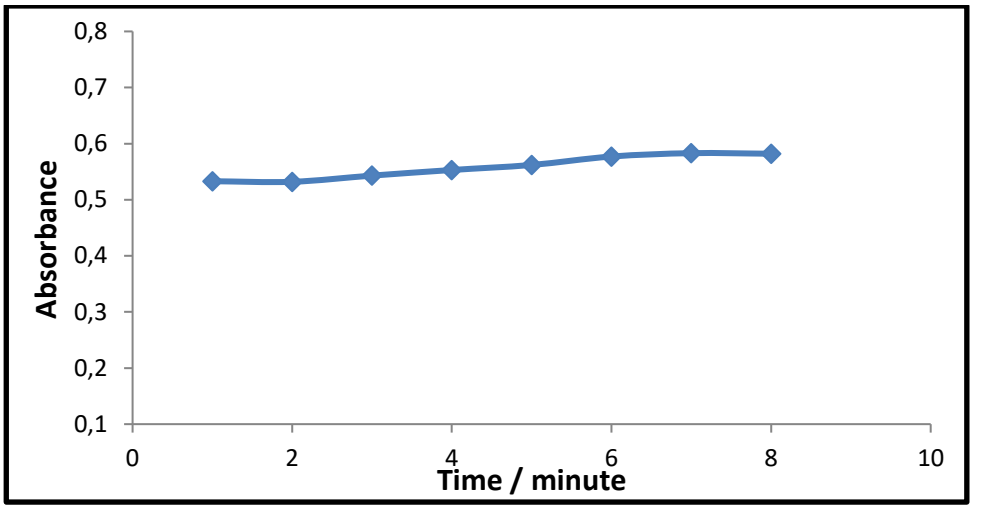


Fig. 3: Effect of time on the coupling reaction

Fig.3 reveals that the coupling reaction between the diazotized CFX and CRO-A reagent needed to 6 minutes to completed after mixing.

Effect of alkaline solution

We studied this state by using strong and weak bases and deferent amounts from each one.The results illustrations in (Fig. 4).

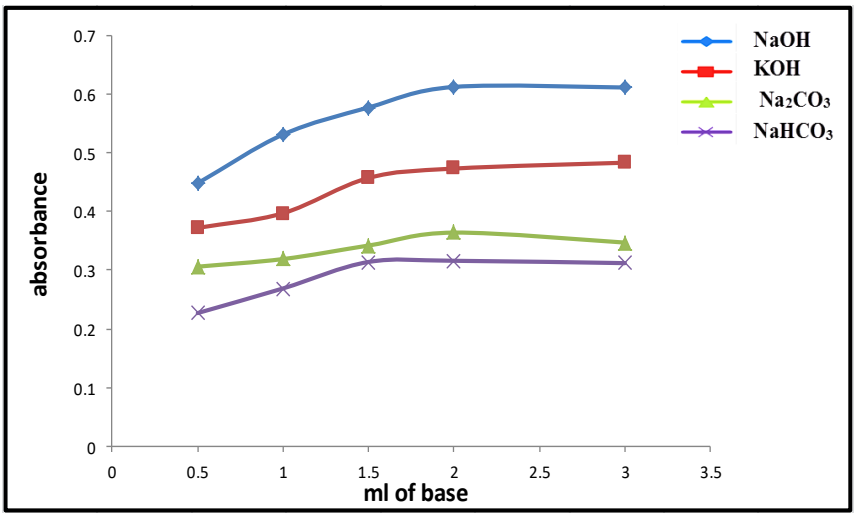


Fig. 4: Effect of alkaline medium

The data of Fig. 4 shows that 2 ml of 1M NaOH give maximum absorbance and more sensitive . The azo dye has good stability. Therefore, it was selected to use in the following experiments.

Effect of surfactants on absorbance of azo dye

Four types of surfactants (CTAB, SDS, Triton X-100 and Tween) were added to the method mixture which revealed no improvement in the sensitivity of the proposed method. The strength of azo dye. Therefore their use in the subsequent experiment was excluded.

Stability of the azo dye

Under optimum conditions, and at 514 nm by preparing two different amounts of 100 and 200 µg CFX. Absorption was measured at various times up to 60 minutes. The Fig. 5 shows that the absorption of the azo dye reaches its maximum and the absorption remains stable at room temperature for at least 60 minutes.

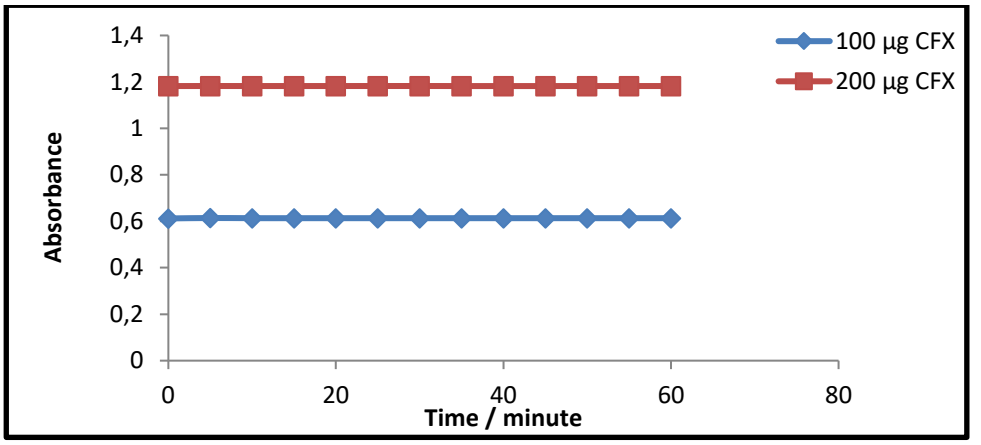


Fig. 5: The stability of azo dye

Final Spectrum

The orange azo-dye formed between diazotized 100 µg of CFX /10 ml and CRO-A in presence the components of the proposed method shows a maximum absorption at 514 nm, while the blank gave a slight absorption at same wavelength (Fig. 6).

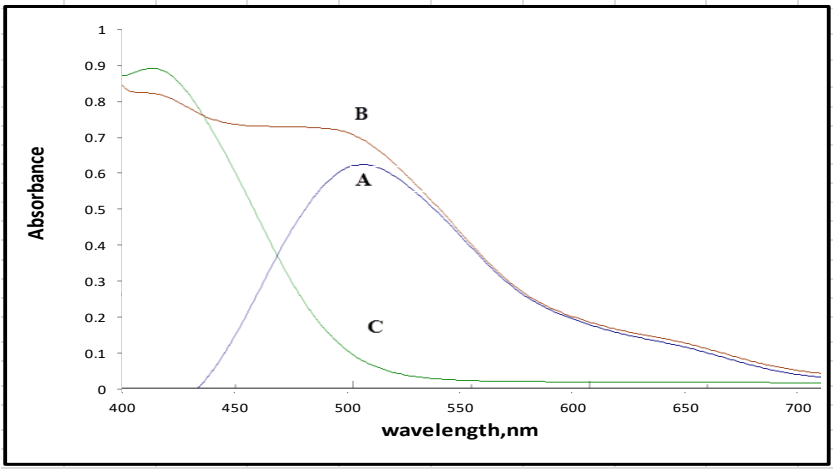


Fig. 6: Absorption spectra of 100 ppm CFX treated according to the recommended procedure against (A) blank, (B) distilled water and (C) blank measured against distilled water

Recommended procedure and calibration curve

At optimum conditions we add 0.1-4 ml of 100 µg/ml pure CFX solution in a series of 10 ml volumetric flasks, equivalent amounts of $NaNO_2$ solution and 1ml of 1 M hydrochloric acid solution. The contents were carefully mixed and kept at 5 C^o for 5 minutes before adding 2.0 ml of 1% CRO-A reagent and 2 ml of 1M sodium hydroxide solution and waiting for 6 minutes before complete the volume to the mark with distilled water. The absorbance of the resulting azo dye was recorded at 514 nm against the blank solution. Linear relationship between CFX uptake and concentration was obtained between 5 and 300 µg CFX/10 ml (Figure 7). The apparent molar absorption coefficient and Sandel sensitivity were 2.892×10^4 L/mol.cm. 0.0175 g/cm^2 respectively.

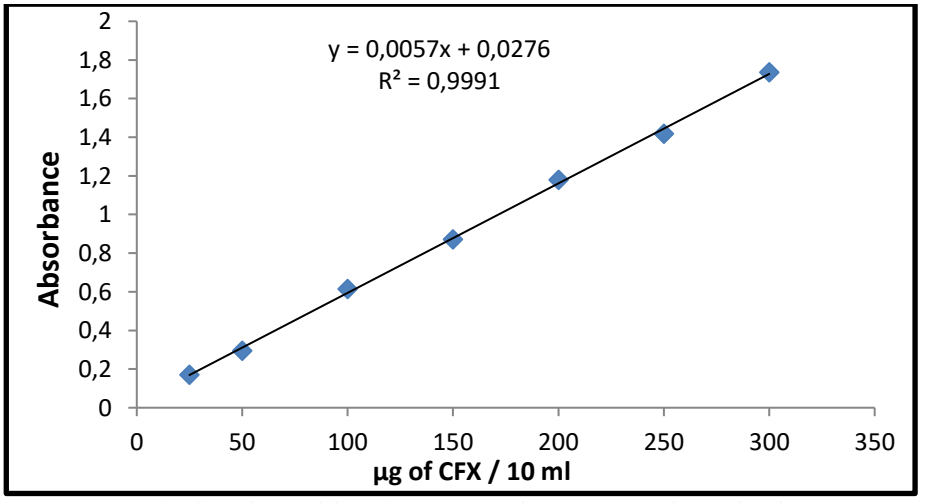


Fig. 7: The calibration curve for proposed method

The nature of the azo dye formed

The stoichiometry for final product was Investigated under optimal conditions by applying the continuous changes (job) and molar ratio methods (Delevie, 1997). The results obtained in Fig. 8 a and b.

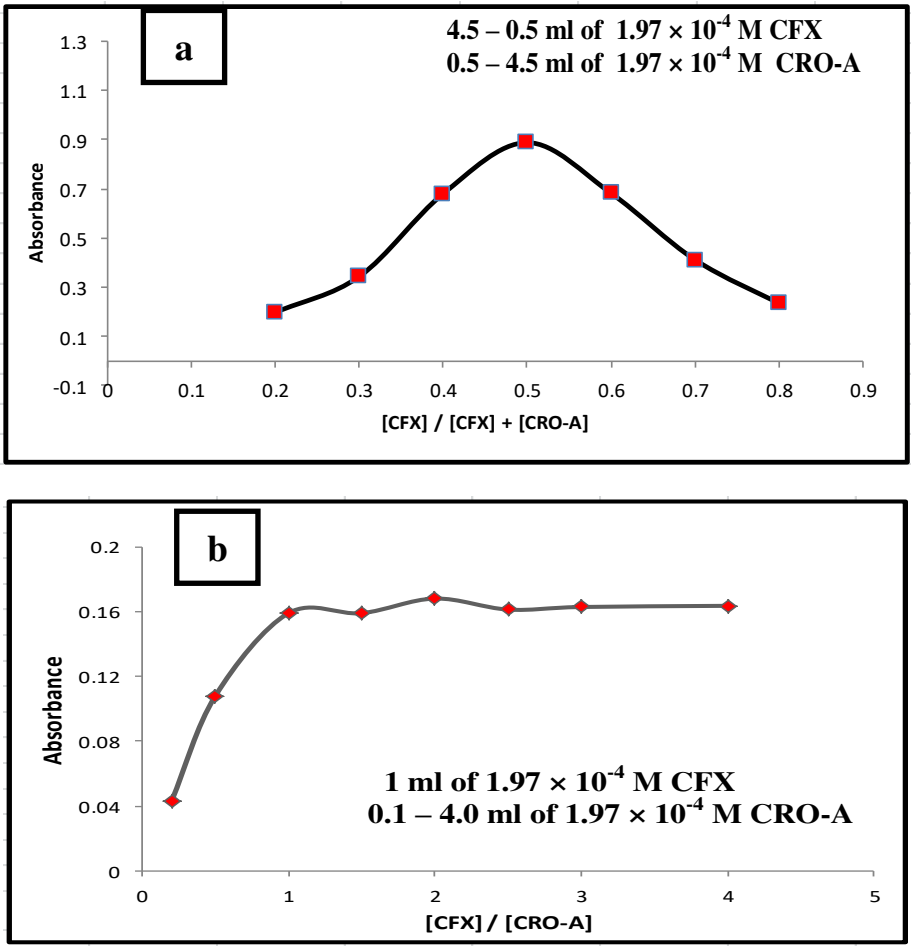
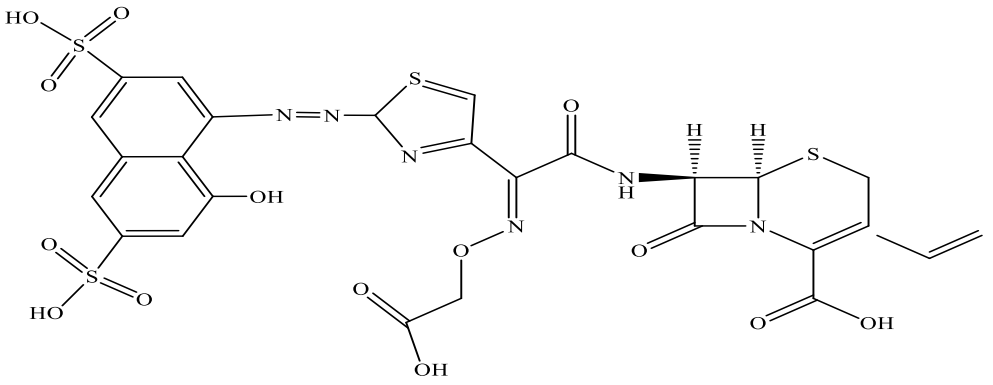


Fig.8: stoichiometry of the azo dye by (a) A continuous variation and (b) mole ratio plots for coupling between CFX and CRO-A under the optimum conditions

Fig.8 shows that the azo dye was formed by a 1:1 combination ratio of diazotized CFX with CRO-A. Based on the results obtained in Fig. 8 we can be expressed the structure of the azo dye as follows Scheme 3.



Scheme 3: The composition of orang azo dye

Effect of interference

Using the recommended procedure the effects of some additives (excipients) that commonly found in pharmaceuticals was studied by adding different amounts of these compounds to 100 µg of CFX. the results are shown in (Table 2).

Table 2: Effect of interference on absorbance of azo dye

foreign compound	Recovery (%) of 100 µg CFX / µg of foreign compound added		
	100	200	300
Glucose	99.85	98.12	97.27
Lactose	99.34	99.08	98.55
Starch	98.79	98.05	97.14
Gum Arabic	99.94	99.01	98.58
Talc	100.09	99.87	99.06
Sorbitol	100.12	99.68	98.76

From the results of Table 2, we find that the studied additives have little effect on the results of the proposed method.

Method applications

To test the suitability of the suggested method for the determination of CFX in some drugs (tablets) we applied our technology to these drugs. We got the results as presented in (Table 3):

Table 3: Application of the method

pharmaceutical preparation	CFX Present (µg)	CFX Found (µg)	Relative error (%)*	Recovery (%)*	RSD*
winex tablets 200 mg CFX/tablet. (K.S.A)	20	.2011	0.55	100.55	1.76
	50	50.84	1.68	101.68	0.93
	100	.9978	-0.22	99.78	1.18

	200	196.69	- 1.65	98.34	2.77
suramix tablets 400 mg CFX/tablet (Jordan)	20	20.89	4.45	104.45	0.78
	50	49.66	-0.68	99.32	1.43
	100	98.85	- 1.15	98.85	3.08
	200	194.97	- 2.51	97.48	2.12

*average of four determinations

Evaluation of proposed method

In order to verify the effectiveness and selectivity of the proposed method, we applied the standard addition method to the available pharmaceutical preparations. The results in Figure 9 and Table 4 showed that the recommended method can be successfully applied to determine CFX without significant interference in the estimation process.

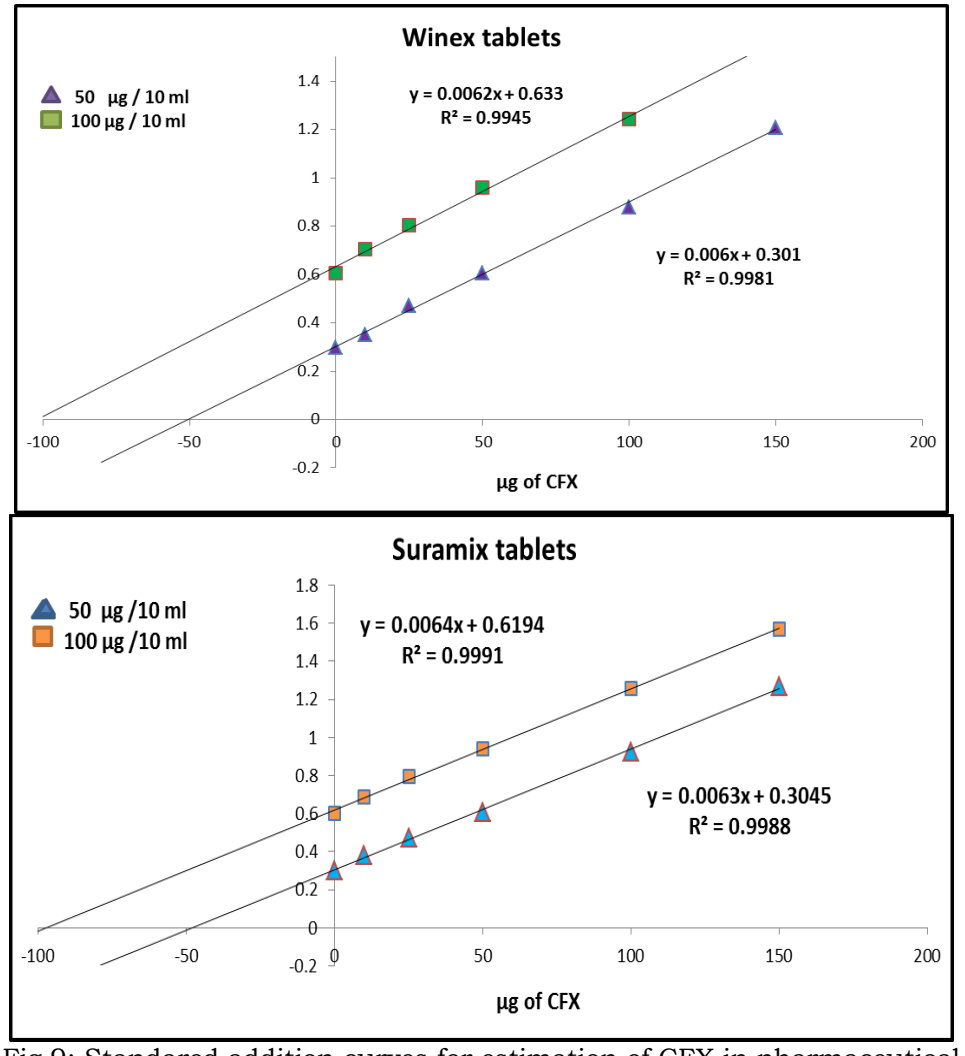


Fig.9: Standared addition curves for estimation of CFX in pharmaceutical preparations

Table 4:The results of standard addition methods for assay CFX

pharmaceutical preparation	CFX Present (µg)	CFX Measured (µg)	Recovery(%)
winex tablets 200 mg CFX/tablet. (K.S.A)	50	50.16	100.33
	100	102.09	102.09
suramix tablets 400 mg CFX/tablet (Jordan)	50	48.33	96.66
	100	96.78	96.78

Conclusion

A spectrophotometric technique using diazotization reaction for the dedication of CFX has been proposed. The method exceedingly selective and sensitive and it characterized by simplicity such as extraction steps and the method has been successfully carried out to pharmaceutical preparations with desirable accuracy and precision.

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