Spectrophotometric Determination of Ciprofloxacin by Ion-pair complex Formation with Bromothymol Blue

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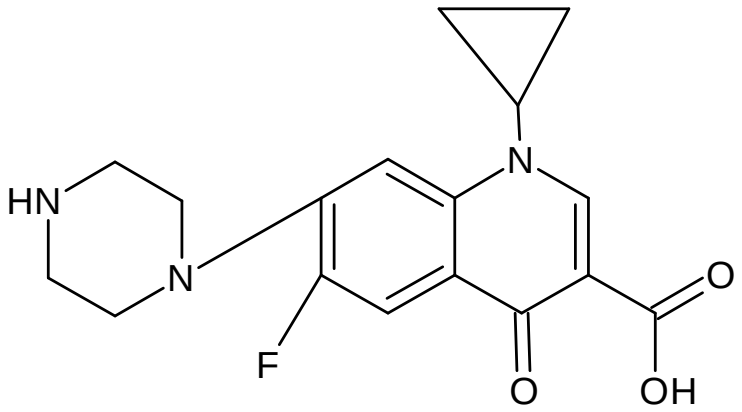
ABSTRACT

A new simple spectrophotometric method based on the formation of an ion –pair using bromothymol blue as ion –pair complexion reagent was developed for the determination of ciprofloxacin in pure form and tablets . Theion-pair formed was highly colored and easily extracted with chloroform at (pH3.0) and determined spectrophotometrically at 421 nm .The proposed method was successfully applied to determine (CPF) in its tablet formulation and the results are good , No interferences from an effective addition were noticed .

Introduction:

Fluoroquinolone antibiotics such as ciprofloxacin (CPF) [1-Cyclopropyl- 6- Fluoro-1, 4- Dihydro - 4 – Oxo -7 (1-Piperazinyl) -3 - Quinoline Carboxylic Acid] is synthetic ,orally active , broad-spectrum agent effective against resistant mutants of bacteria[1-2]



Structure of Ciprofloxacin[2]

It is also reported that there is a direct correlation of flu oroqinolone bonding inhibition of fluorquinolone one bonding with inhibition of DNA-

gyrase enzyme activity and induction of DNA breakage. Because of this special mechanism of action , fluoroquinolones are considered to be the most effective gram –positive – gram- negative pathogens to combat infections caused by micro organisms that are resistant to other microbials, such as tetracycline .The drug is official in British pharmacopoeia[3] and united states pharmacopoeia[4] which describe a high performance liquid chromatographic (HPLC) method for its assay.

Several methods have been reviewed in the literature for the analysis of ciprofloxacin the USP[5] recommends a liquid chromatographic method for determination of ciprofloxacin and anon-aqueous titrimetricmethod.

Spectrophotometric methods reported for the determination of ciprofloxacin included ion-pair complex formation with cobalt (II) Tetrathiocyanate[6] , as well as complex with Bismuth (III) Tetraiodide[7] other method include titrimetry using cerium (IV) sulphate[8].

UV-spectrophotometry has also been used for the assay of CPF in single dosage forms[9] and in two component mixture[10-11] , charge-transfer complexation[12-13] and ion-pair complexation[14-15] reactions are found in the literature for the assay of CPF in formulations .

The aim of the present work is to develop a new spectrophotometric method for determination of ciprofloxacin in pharmaceutical preparations .For this purpose we studied the formation of ion-pair between the ciprofloxacin and Bromothymol Blue (BTB), and the possibility for its extraction and determination in this way .

Experimental:

- **Apparatus**

Acintra 5 spectrophotometer with 1 cm quartz cells was used for absorbance measurements.

pH-meter DW-9421 from Philips instrument, a Sartorius BL 210S balance, and a Pentium 4 computer (DELL)was used for data processing .

Materials and Reagents

- **Stock solution of (CPF)**

A pure powder of ciprofloxacin was kindly provided by (SDI). Stock solution of ciprofloxacin was prepared (20 µg/mL) by dissolving (0.01 gm) pure drug in (100 ml)distilled water .

- **Reagent Solution**

A stock solution of BTB dye (Merck)(0.05%) was prepared by dissolving (0.05 mg) of BTB in (5ml) methanol and then diluted to (100 ml) with distilled water .

Working solutions were freshly prepared by subsequent dilutions with distilled water .

- **Buffer solution**

Phthalate buffer (pH3.0) was prepared by dissolving amount of potassium hydrogen phthalate in distilled water .

Preparation of calibration curve:

Standard solutions of (CPF) having final concentration in the range (0.5 - 30) ppm were transfered (1ml of each)in to separatory funnels ,(1ml)of BTB solution (0.04%) and (0.5ml) of phthalate buffer solution (pH3) were added to each .The reaction mixture was extracted by shaking for (2min)with (5ml) of chloroform .The absorbance of the organic layer was measured at (421nm) against blank reagent and the measured values were plotted against CPF conc . (Figure 2).

Procedure for Tablets:

The content of (10) tablets were mixed well and a certain amount of fine powder was accurately weighted to give an equivalent to 500 mg was dissolve in 10 ml of distilled water and diluted to 100 ml in a volumetric flask with distilled water .The solution was filtered by using whatman filter paper No .41.working solutions were freshly prepared by subsequent dilutions with distilled water .

Results and Discussion

- **Effect of pH**

The influence of pH on the ion-pair formation of (CPF) with BTB has been studied using phthalate buffer .The results showed in the range (2-5) that the most efficient extraction of the ion pair with chloroform was obtained at a pH of (3.0) The results are shown in (Figure 3).

Effect of order of addition of reactants

The sequence of order of addition of the reactants had shown that order should be ciprofloxacin ,phthalate buffer and BTB (Table1).

Order of addition of reactants			A
D	B	R	0.7864
D	R	B	0.5889
B	R	D	0.6693

Table 1. Effect of Order of addition of reactants D(Drug), B(Buffer), R(Reagent)

Effect of reagent concentration

The BTB concentration suitable for the ion-pair formation and extraction was found to be (0.04 %) show in (Fig 4).

Effect of shaking time

The effect of the shaking time on the extraction of the CPF-BTB ion-pair was studied . shaking times ranging (from 1 to 10 min) produced that the best time is 2 min (Table 2).

Time/mt.	A
1	0.7020
2	0.7864
3	0.7453
4	0.7426
5	0.6998
10	0.6263

Table 2 . Effect of shaking time

Effect of organic solvent

Different solvents have been tried in order to a chieve maximum sensitivity and product stability, chloroform, Tetra chlorocarbon, 1,2 di chloro methane , Benzen and cyclohexan.

It was found that ion-pair complex was readily and quantitatively extractable in chloroform .

Therefore chloroform was used as extracting solvent (Table 3).

Solvent	A
Chloroform	0.7864
Carbon Tetra Chloride	0.0334
Benzene	0.0790
1,2Dicloro Methane	0.5723
Cyclohexane	0.6017

Table 3 . Effect of organic solvent

Stoichiometric Relationship

The composition of the ion-pair associates was established by Job's method of continuous variation[16-17] using equimolar solutions of the CPF and BTB The results obtained are shown in (Figure 5) .

The results indicated that the complex was formed in the ratio of 1:1 .

This stoichiometric ratio supports that the interaction of the CPF and BTB takes at only one site which was the more sterically free terminal basic aliphatic amino group [18].

Application:

The proposed method was successfully applied to determine ciprofloxacin in its commercial tablets.

The results are presented in Table 4.There was no evidence of interference from excipients in the tablets analysed.

Sample	Amount Labeled (mg)	Taken Conc. (µg. mL^{-1})	Found Conc.* (µg. mL^{-1})	% Recovery	%R.S.D*
ciprofloxacin 500mg/ tablet Sirya	**500**	**5**	**5.283**	**105.660**	**1.546**
		20	**21.399**	**106.995**	**1.989**
ciprofloxacin 500mg/ tablet EUA	**500**	**5**	**5.136**	**102.72**	**1.472**
		20	**20.845**	**104.225**	**1.867**

Table 4.Application

Effect of Interference

The proposed method was applied with found number of interference in pharmaceutical formulations , No interference was observed from commonly used excipients , the results are presented in Table 5.

Excipients	Ciprofloxacin Conc. Taken (20 µg.mL^{-1})	
	Conc. Found (µg.mL^{-1})	%Recovery
Magnesium Stearate	19.779	98.895
Sodium Citrate	20.371	103.940
Starch	19.788	98.940
Glucose	19.862	99.310
Lactose	20.666	103.330

*Average of three determinations.

Table 5 . Effect of interference

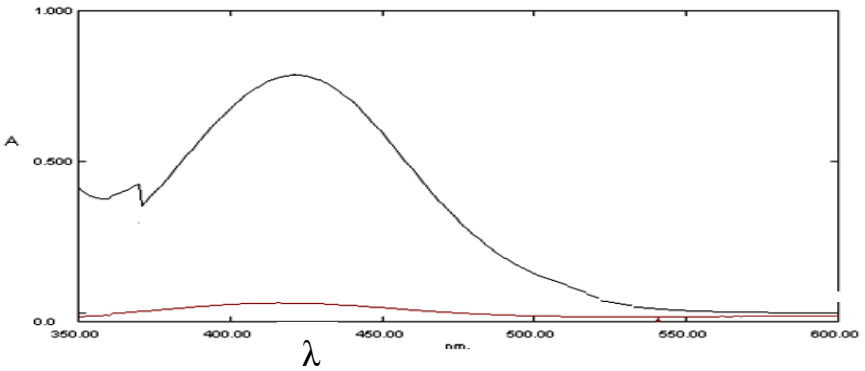


Figure 1. The spectrum of 20 µg/ml of ciprofloxacin against blank and the reagent blank against chloroform

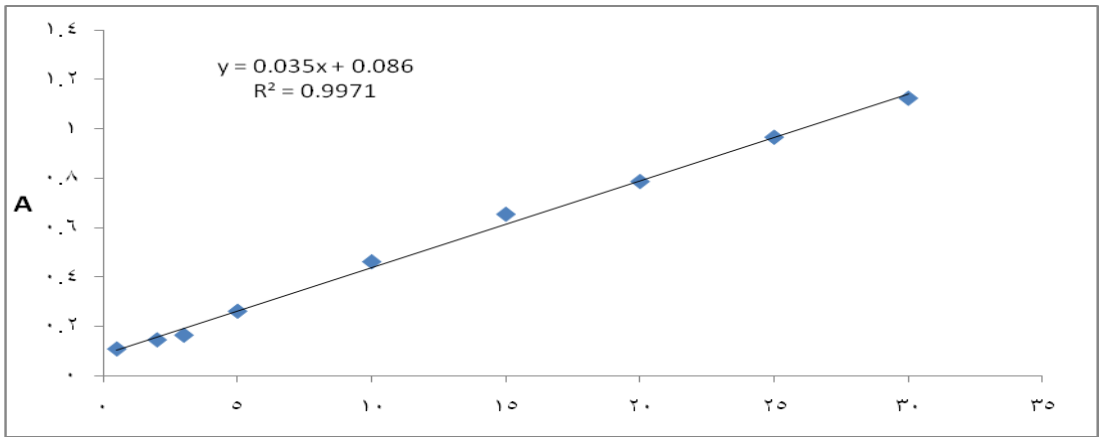


Figure2. Calibration curve

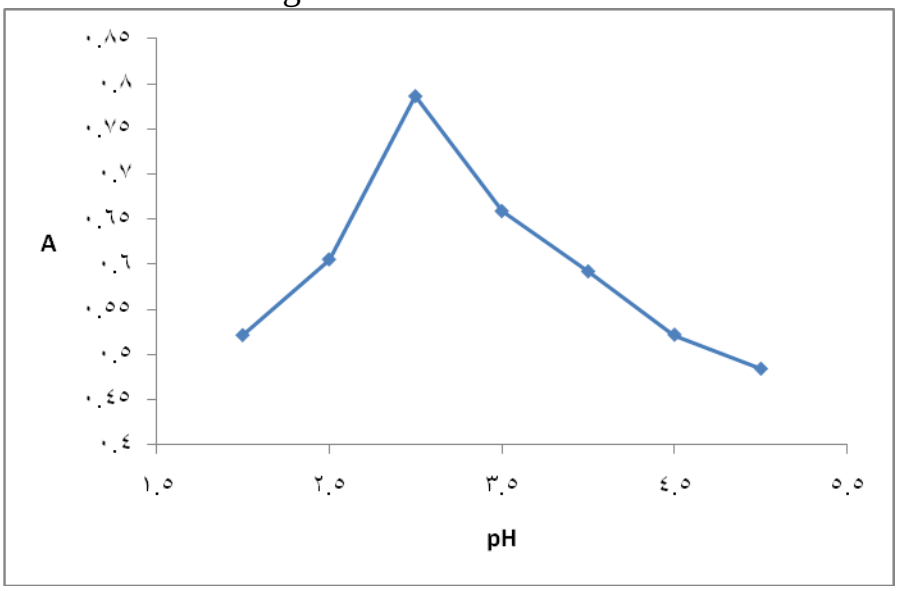


Figure 3. Effect of pH

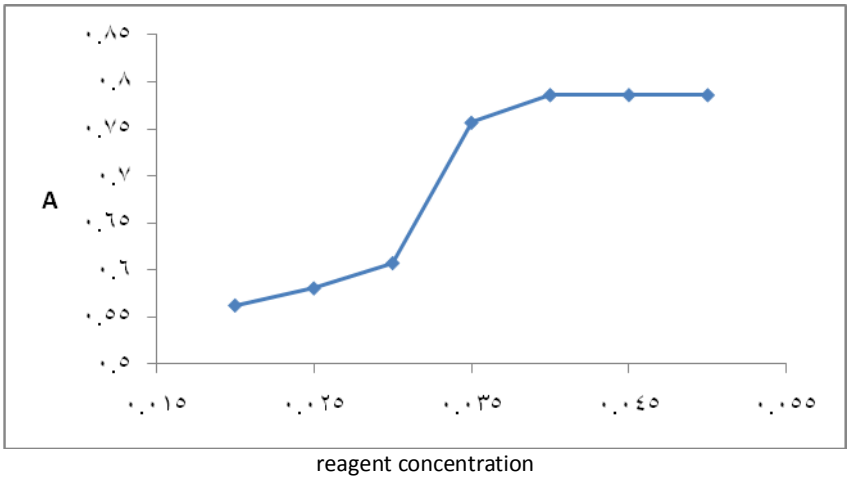


Figure 4. Effect of Reagent concentration

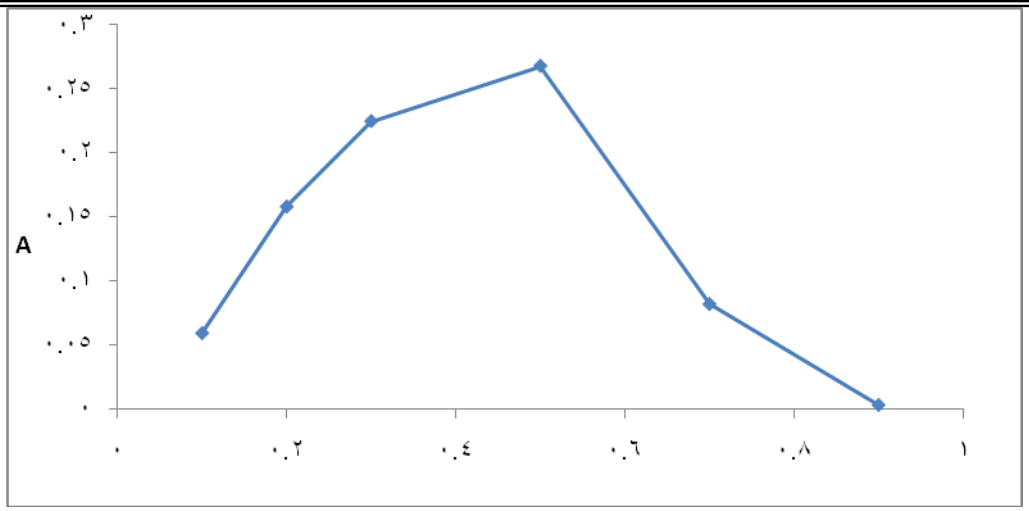


Figure5. Job's method

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الخلاصة

تهدف الدراسة الى تقدير (CPF) على هيئة مسحوق نقي و هيئة اقراص سوري و اماراتي) بطريقة طيفية بسيطة تعتمد على تكوين معقد من نوع مزدوج ايوني باستعمال (BTB) ككاشف للمعقد الايوني .

ان المعقد الايوني المتكون شديد اللون و سهل الاستخلاص باستعمال الكلورفورم عند (pH3.0) و يتم تقديره طيفيا عند طول موجي (421 nm) .

و قد لوحظ ان الطريقة المقترحة طبقت بنجاح لتقدير (CPF) على هيئة اقراص و كانت النتائج جيدة , كذلك لوحظ انه لا يوجد اي تداخلات من قبل الاضافات غير الفعالة .