SPECTROPHOTOMETRIC DETERMINATION OF NAFCILLIN SODIUM IN PURE FORM, TABLETS AND BIOLOGICAL FIUIDS

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تم وصف اثنين من الطرق الطيفية البسيطة والحساسة لتقدير مركب نافسيلين الصوديوم في صورته النقية وفي الأقراص الدوائية وفي سوائل الجسم الحيوية. الطريقة الأولى تعتمد على اختزال ايونات الحديد الثلاثي إلى الحديد الثنائي في وجود كاشف الأورثوفينانثرولين بواسطة نافسيلين الصوديوم حيث يتكون معقد الفيروين ذو اللون الأحمر البرتقالي على درجة عالية من الثبات والذي يمتص عند طول موجي ٥١٠ ناتومتر، ويكتمل تكون اللون بالتسخين. أما الطريقة الثانية فتعتمد على تفاعل نافسيلين الصوديوم كمانح للالكترونات مع كاشف باراحمض الكلورانيل كمستقبل للالكترونات حيث يتكون معقد ملون يقاس امتصاصه عند ٥٣٠ نانومتر. تم تطبيق قانون بيير والحصول على علاقة خطية في مدى من التركيز لنافسيلين الصوديوم بين ١,٢٥-١٥ ميكرو جرام / مل و ١٥-٢٠٠ ميكرو جرام / مل وبحد كشف مقداره ٠,١٢ ميكرو جرام / مل و ٣,٦١ ميكرو جرام / مل، ومعامل ارتباط خطي يساوي ٠,٩٩٩٩ (لعدد ٩ عينات) و ٠,٩٩٩٨ (لعدد ١٠ عينات) لكل من الطريقتين على التوالي، ووجد أن متوسط الانحراف المعياري النسبي للنتائج كان أقل من أو يساوي ١,٩ % مما يدل على تكرارية جيدة لهاتين الطريقتين. وقد تضمن البحث ظروف التفاعلات المثلى التي تم التوصل إليها مع إمكانية تطبيقها لتقدير المركب المذكور في الأقراص الدوائية. وقد أظهرت الدراسات الإحصائية توافقاً للنتائج مع تلك المتحصل عليها من طريقة منشورة استخدمت للمقارنة كما أظهرت الطريقة الأولى إمكانية تطبيقها بنجاح على سوائل الجسم الحيوية كالبول والبلازما لتقدير نافسيلين الصوديوم فيها.

Two simple and sensitive spectrophotometric methods are described for the determination of nafcillin sodium in pure form, tablets and biological fluids. The first method (method 1) is based on the reduction of ferric ions into ferrous ions in presence of *o*-phenanthroline by nafcillin sodium to form a highly stable orange-red ferroin chelate $[Fe - (phen)_3]^{2+}$, measured at 510 nm. Maximum color formation was obtained through heating. The second method (method 2) is based on the reaction of nafcillin sodium as n - donor with *p*-chloranilic acid (*p*-CA) as a π-acceptor to form an orange - red complex measured at 530 nm. Bear's law is obeyed in the ranges of 1.25-15 μg ml^{-1} and 15-200 μg ml^{-1} with limits of detection of 0.12 μg ml^{-1} and 3.61 μg ml^{-1} and correlation coefficients of 0.9999 (n = 9) and 0.9998 (n = 10) for methods 1 and 2, respectively. The mean relative standard deviations (RSD%) of the results within day precision and accuracy of them were ≤ 1.9 % which confirmed the reproducibility of the assay technique. The optimum assay conditions and their applicability to the determination of the drug in tablets are described. The methods results showed insignificant difference with those of a reference method. The first method was successfully applied to the determination of nafcillin sodium in spiked urine and plasma.

Keywords: Spectrophotometric; Nafcillin sodium; Tablets; Biological fluids; Ferric-phenanthroline complex; *p*-Chloranilic acid.

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INTRODUCTION

Nafcillin sodium (I) is a semisynthetic β-lactamic antibiotic widely used in medicine as an effective way of healing serious infections caused by staphilococus aureus due to its resistance to penicillinase produced by these bacteriums; therefore, the determination of nafcillin is extremely important [1,2]. Numerous analytical procedures have been reported for its determination in pure form, pharmaceutical preparations or in biological fluids. The USP pharmacopoeia [3] recommends an HPLC assay, for the evaluation of its raw material and dosage forms. Other reported methods for determination of nafcillin are based on different analytical techniques including HPLC [4-7], HPLC / MS [8,9], LC [10-12], ion-pair LC [13], LC/MS [14,15], GC [16,17], MS [18], phosphorimetry [19,20], fluorimetry [21-23] and spectrophotometry [24,25]. The present paper describes two new, sensitive, accurate and rapid spectrophotometric methods for the determination of nafcillin sodium in pure form, tablets and biological fluids.

EXPERIMENTAL

Apparatus:

A UV-VIS Spectrophotometer (Ultrospec 2100 pro/80-2112-21/Amersham Bioscience) with quartz cells of 1.0 cm path length was used for the λ_{max} determinations and all absorbance measurements.

Reagents and Materials:

Analytical reagents grade chemicals, solvents of spectroscopic grade and double distilled water were used throughout.

Double pure nafcillin sodium sample was provided by SIGMA Chemical Co., USA and used as received. 125 μg ml^{-1} drug standard solution was prepared in distilled water for method-1 and 500 μg ml^{-1} drug standard solution was prepared in methanol for method 2. Working standard solutions were prepared by appropriate dilution immediately before use. Tablets containing the studied drug were prepared as each tablet containing 250 mg of nafcillin sodium and the tablet excipients: lactose (350 mg), starch (30 mg), magnesium stearate (6.5 mg) and talc (31.5 mg) per tablet [26]. Ferric *o*-phenanthroline mixture (Fe^{3+} - phen) was prepared by transferring 0.2 g of *o*-phenanthroline (Riedel de Häen Co., Germany, 99.5%) into a 100 ml volumetric flask, 2 ml of concentrated hydrochloric acid (BDH, 38%) and 0.16 g of $Fe(NO_3)_3.9H_2O$ (GRG) were added and the volume was completed to the mark with distilled water. A buffer solution of pH = 5.08 was prepared using 0.2 M of acetic acid (BDH) and 0.2 M of sodium acetate (BDH). 0.02 M *p*-Chloranilic acid (*p*-CA) was prepared by dissolving 0.417g of *p*-CA (MERCK) in methanol (BDH) in a 100 ml volumetric flask. Plasma (UDITROL"N"), acetonitrile (MERCK) were used and urine samples were obtained from healthy volunteers.

Procedures:

Recommended procedures for calibration:

For method 1:

Working solutions of different volumes (0.1 -1.2 ml) of standard stock solution of nafcillin (125 μg ml^{-1}) were transferred into a series of 20 - ml test tubes. 1.5 ml of (Fe^{3+} - phen) mixture and 3 ml of acetate buffer solution (pH 5.08) were added to each tube and shaked. The test tubes were heated in a water bath at 80°C for 30 min, cooled to room temperature and transferred, quantitatively the contents of each tube to a 10 ml volumetric flask and the volume was completed with water. The absorbance of the resulting orange-red solution is measured at 510 nm against a blank solution treated similarly. Calibration graph was prepared by plotting the absorbance against the drug concentration over the ranges cited in Table 1 for nafcillin sodium.

For method 2:

Working solutions of different volumes in the range (0.4 - 4 ml) of standard stock solution of nafcillin (500 μg ml^{-1}) were transferred into a series of 10 ml volumetric flask. The drug sample is allowed to react with 1 ml of 0.02 M *p*-CA and the orange-red complex was produced immediately. The volumes were completed with methanol and the absorbance of each solution was measured at 530 nm against a reagent blank solution. Calibration graph was prepared by

plotting the absorbance against the drug concentration over the ranges cited in Table 1.

Procedure for tablets:

Ten tablets were mixed and weighed. An accurately weighed amount of the powder equivalent to 12.5 mg of nafcillin sodium was transferred into a beaker with 50 ml distilled water, sonicated for 5 min and filtered into a 100 ml volumetric flask, then completed to volume with water to obtain a 125 μg ml^{-1} standard solution. The above procedure for method 1 was then followed. The nominal content of the tablets was calculated from the corresponding calibration graph or using the regression equation. Also, an amount equivalent to 25 mg of the drug was weighed accurately and transferred into a 100 - ml beaker with 50 ml methanol, sonicated for 5 min and filtered into a 50 ml volumetric flask. The volume was completed with methanol to get a 500 μg ml^{-1} standard solution for method 2. The method of standard additions was followed for the determination of nafcillin in tablets using the procedure described above for method 2.

Procedure for biological fluids:

Procedure for spiked urine:

12.5 mg of nafcillin sodium was weighed accurately and added to 1 ml urine in a 100 ml volumetric flask. The spiked urine was diluted with water to obtain 125 μg ml^{-1} of nafcillin sodium and the procedure for method 1 was then followed. Blank solution was prepared by treating the antibiotic - free urine in the same manner.

Procedure for spiked plasma:

12.5 mg of nafcillin sodium was added to 1ml of plasma followed by 1 ml acetonitrile and centrifuged at 3000 rpm for 10 min. The supernatant was diluted with water to the mark in a 100 ml volumetric flask to obtain a 125 μg ml^{-1} nafcillin sodium solution and the procedure for method 1 was followed against a blank solution similarly treated antibiotic - free plasma.

RESULTS AND DISCUSSION

Method 1:

Different betalactam antibiotics have well - known reducing characters [27,28] which may be due to their sulfur content. This phenomenon was used in this study to determine nafcillin sodium by a spectrophotometric method. This method depends on that a quantity of ferric ions was reduced by the analysed antibiotic into ferrous ions. Then the later reacted with *o*-phenanthroline to yield the orange-red ferroin chelate with λ_{max} 510 nm, Fig.1.

The development of the color depends very much on the reaction conditions. Therefore it is very important to optimize the reaction conditions.

The effect of pH on the absorbance of ferroin chelate was studied over a range of 3.4-5.8. Fig 2 showed that the absorbance at 510 nm increased with increasing pH value up to 5.08, after which it remained constant. From 2-5 ml acetate buffer (pH 5.08) was found to be sufficient for maximum color - intensity.

The effect of volume of (Fe^{3+}/ *o*-phen) reagent was studied in the range 0.5-4 ml. The absorbance increased with increasing volume of reagent. Maximum color intensity was obtained upon using 1.5 ml of (Fe^{3+}/ *o*-phen) reagent as shown in Fig.3.

Temperature greatly enhances the reaction. Different temperatures were tested, from 30-100°C, using the water bath. 75-100°C gave maximum color intensity, 80°C was chosen as the best temperature for the reaction as shown in Fig. 4. The development of the color of the product was completed after 30 min in the water bath at 80°C and remained stable for at least 24 hr. Application of Job's [29] and molar ratio methods [30] indicated a molar ratio of donor to acceptor 1: 4 for nafcillin sodium (Figs. 5,6). The reaction pathway can be represented as shown in scheme 1.

Method 2:

π-acceptors react with basic nitrogenous compounds as n - donors to form charge transfer complexes or radical anions according to the polarity of the solvent used [31]. The amino group in nafcillin represents the electron donor group which is responsible for the formation of charge transfer complexes with electron acceptors.

Nafcillin sodium reacts with p-chloranilic acid (*p*-CA) forming an orange red colored product which exhibit absorption maxima at 530 nm, Fig. 7.

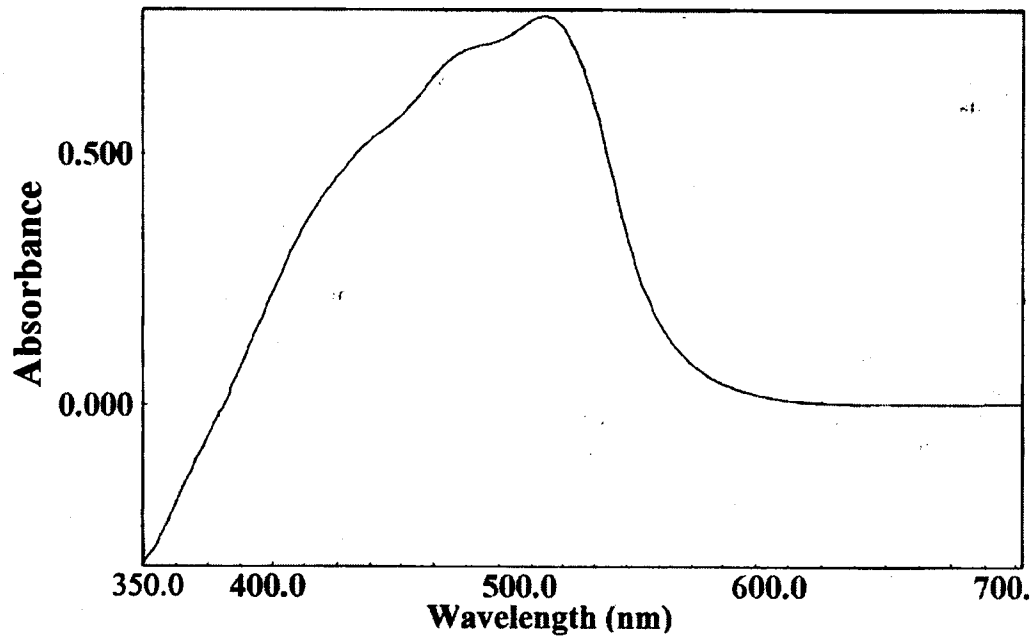


Fig. 1: Absorption spectrum of ferroin chelate formed from the reaction of nafcillin sodium (10 μg ml^{-1}) with 1.5 ml of (Fe^{3+} / *o*-phen) in presence of buffer solution of pH 5.08 after heating at 80°C for 30 min .

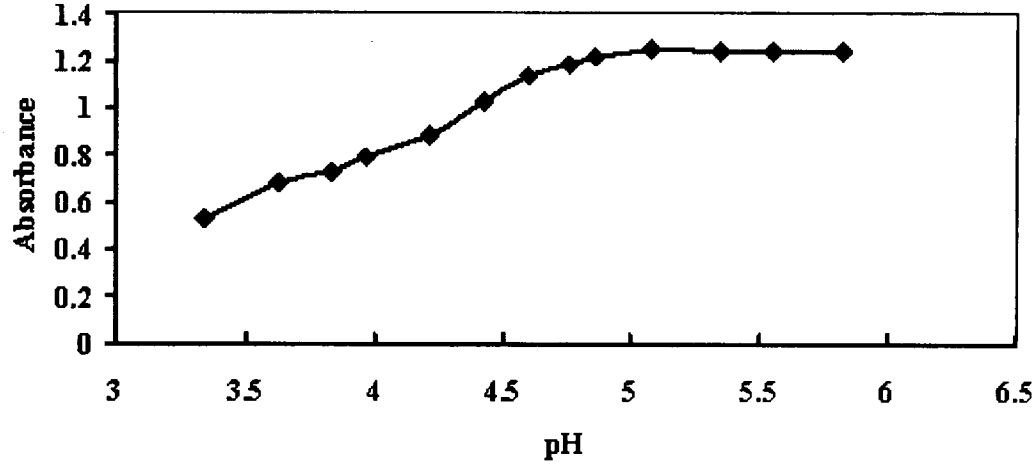


Fig. 2: Effect of pH of buffer solution on the absorbance of the formed ferroin chelate at 510 nm , nafcillin sodium (10 μg ml^{-1}) , (Fe^{3+} / *o*-phen) 1.5 ml, temperature 80°C, heating time 30 min.

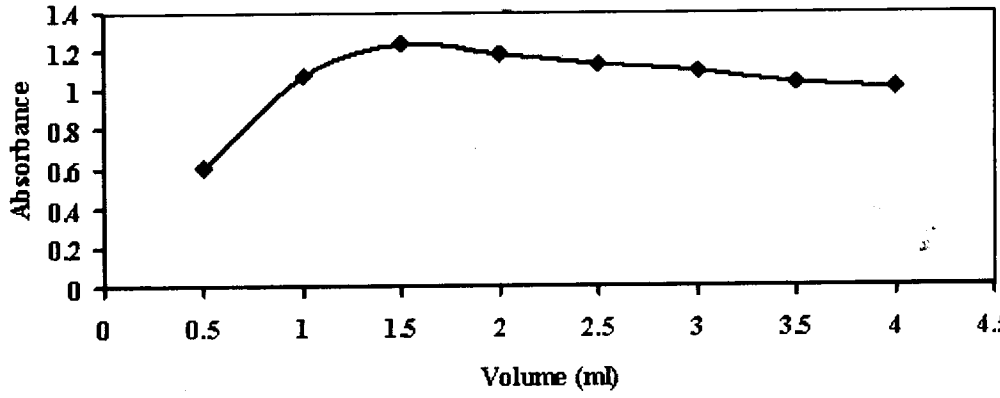


Fig. 3: Effect of volume of (Fe^{3+} / *o*-phen) reagent on the absorbance of ferroin chelate formed at 510 nm , nafcillin sodium (10 µg ml^{-1}) , pH 5.08, temperature 80°C, heating time 30 min.

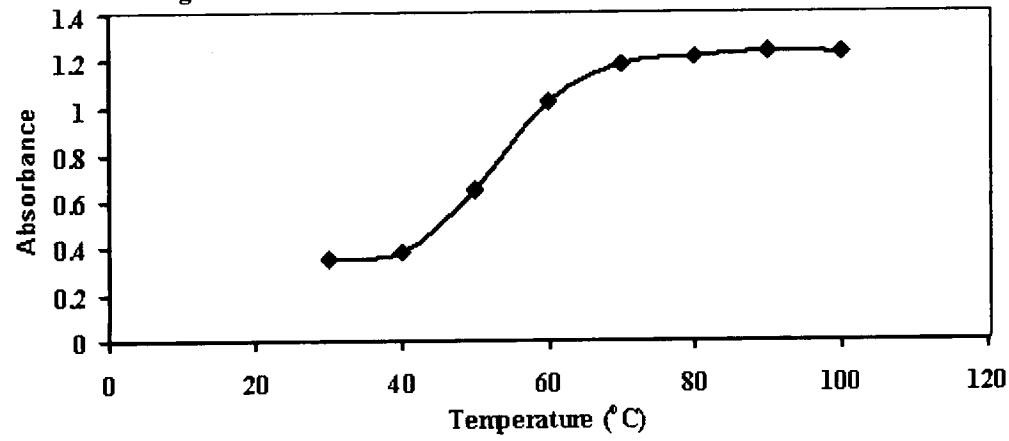


Fig. 4: Effect of heating temperature on the absorbance of ferroin chelate formed at 510 nm, nafcillin sodium (10 µg ml^{-1}), pH 5.08, heating time 30 min.

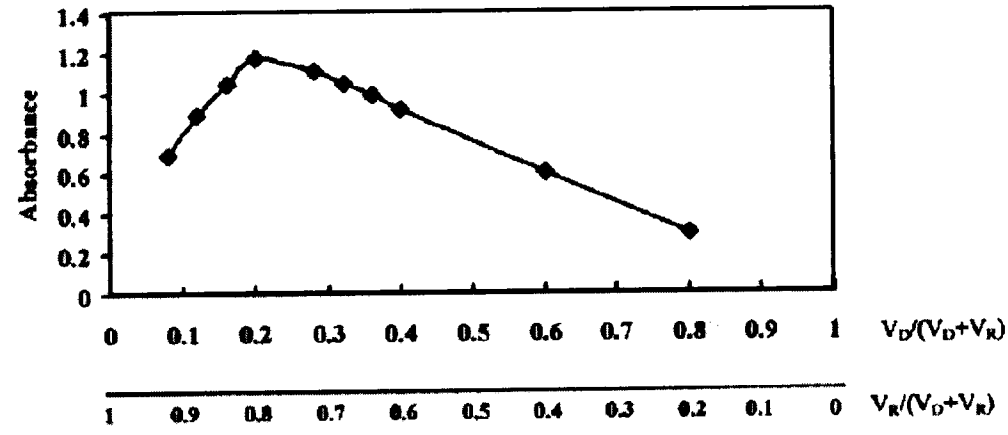


Fig. 5: Stoichiometry of the reaction of (6.154 x 10^{-3} M) nafcillin sodium with (6.154 x 10^{-3} M) Fe^{3+} / *o*-phen by Job's method, V_D = volume of drug, V_R = volume of reagent.

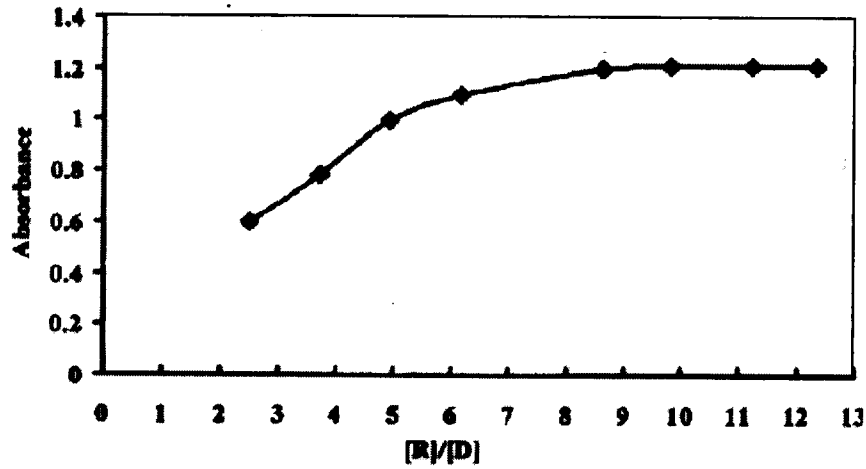
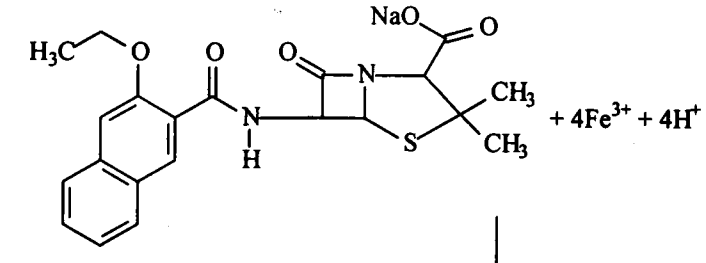
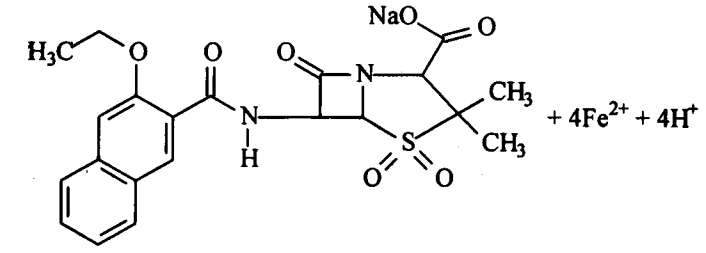
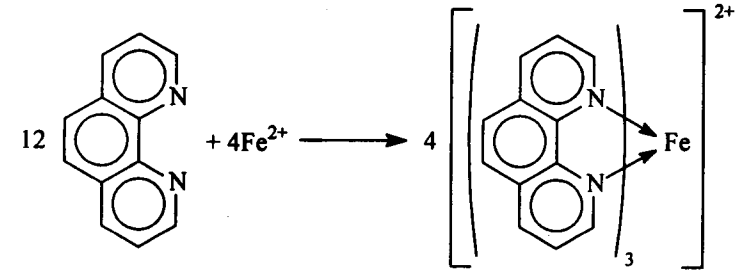


Fig. 6: Stoichiometry of the reaction of (6.154 x 10^{-3} M) nafcillin sodium, [D], with (6.154 x 10^{-3} M) Fe^{3+} / *o*-phen, [R], by Molor ratio method.



Nafcillin Sodium





o-Phenanthroline Ferroin

Scheme (1): Reaction mechanism between Nafcillin Sodium and *o*-Phen/Fe^{3+}

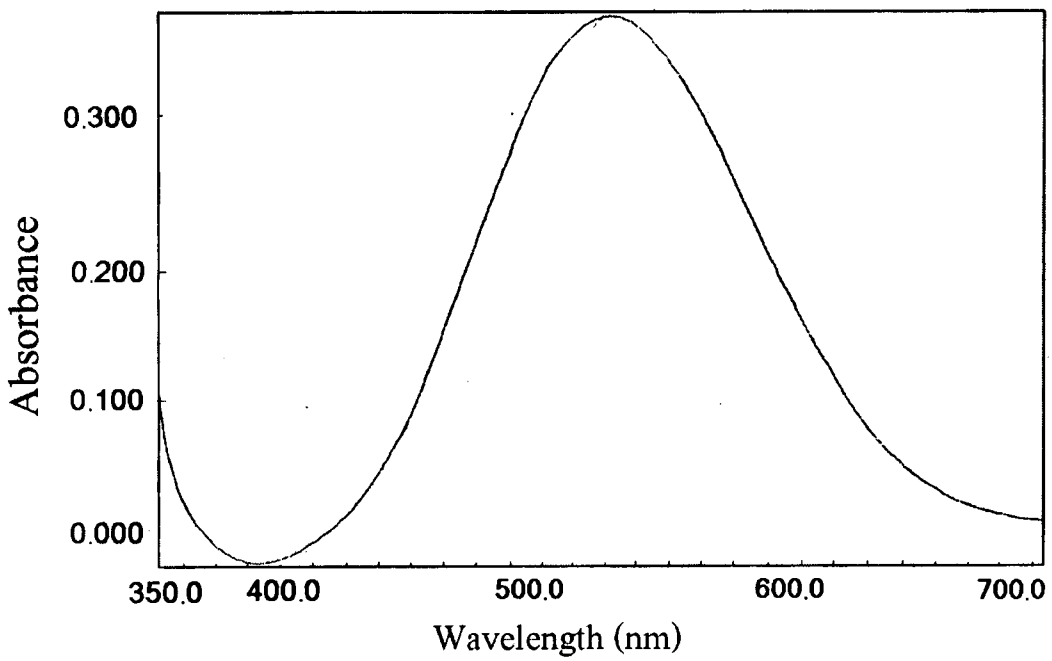


Fig. 7: Absorption spectrum of the reaction product of nafcillin sodium(180 μg ml^{-1}) with *p*-CA (0.002M) in methanol.

Since polar solvent is used (methanol), this band may be attributed to the formation of *p*-CA radical anions [32,33]. The reaction may be represented by the following equation:

$$D^{\cdot\cdot} + A \longrightarrow [D^{\cdot\cdot} \longrightarrow A] \rightleftharpoons D^{\cdot+} + A^{\cdot\cdot}$$

Donor (Nafcillin Sod.) — Acceptor (*p*-CA) — Complex — Radical ions

The reaction conditions were optimized with regard to the nature of the solvent, the concentration of the reagent and the effect of time.

Water, ethanol and methanol were tried as solvents for the color formation and as diluents for the reaction products. Methanol alone was essential for the color stability and quantitative precise results. Ethanol decreases the absorbance of the product and water inhibits the reaction. Methanol is a good solvent for nafcillin sodium and *p*-CA as it affords maximum color intensity and has a fairly good solvating power for π-acceptors.

The effect of *p*-CA concentration was studied. Different volumes of 0.02 M *p*-CA in the range 0.5-3 ml were used. Maximum absorption was obtained upon using 1 ml of 0.02 M *p*-CA with final concentration of 0.002 M of *p*-CA, above which the absorption decreased as shown in Fig. 8.

Maximum color intensity at ambient temperature was attained immediately with *p*–CA and remained stable for more than one hour. Application of molor ratio method [30] indicated a molar ratio of donor to acceptor 1:1 for nafcillin sodium (Fig. 9).

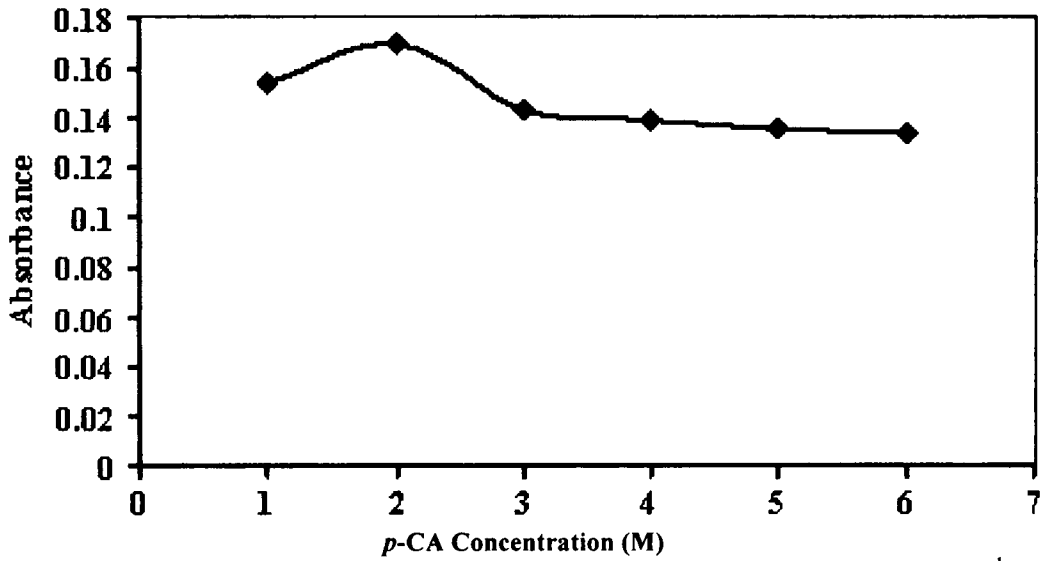


Fig. 8: Effect of *p*-CA concentration on the absorbance of nafcillin sodium (90 μg ml^{-1}) / *p*-CA complex at 530 nm.

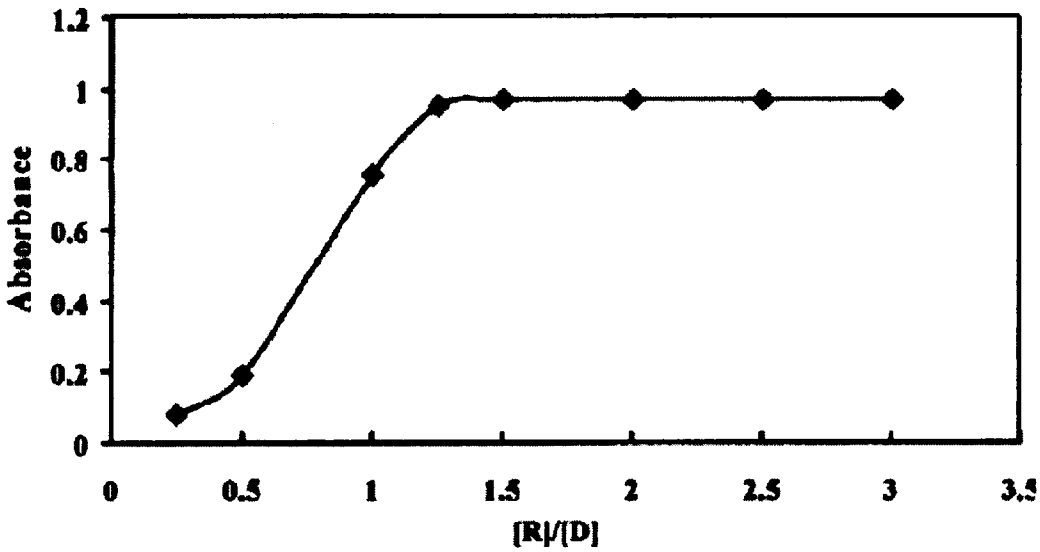


Fig. 9: Stoichiometry of the reaction of (0.01 M) nafcillin sodium, [D], with (0.01M) *p*-CA, [R], by Molar ratio method.

Determination of nafcillin sodium:

Under the described experimental conditions, standard calibration curves for nafcillin sodium by the two methods were constructed by plotting the absorbance versus concentration. Conformity to Beer's law was evident over the concentration ranges of 1.25-15 μg ml^{-1} for method 1 and 15-200 μg ml^{-1} for method 2 as given in Table 1. The relative standard deviations (%RSD) were 0.94% for 10 μg ml^{-1} nafcillin sodium in method 1 and 1.9% for 40 μg ml^{-1} in method 2 based on 10 replicate determinations of each method.

Least - squares regression calibration curves of nafcillin sodium were found to be linear at the studied concentration ranges with correlation coefficients, r, of 0.9999 and 0.9998 for methods 1 and 2 respectively as shown in Table 1. It also shows the linear ranges used, the regression equations parameters, limits of detections and limits of quantitations for both methods 1 and 2.

The precision of the proposed methods was evaluated by analysing standard solutions of the studied drug from replicate analysis (n = 3) at concentrations within the linear ranges of the two methods for nafcillin sodium. The results in Table 2 were in accord with those obtained by a reference method [24].

Statistical analysis [34] of these results using the Paired student's t-test and the variance ratio F-test showed no significant difference between the performances of the methods as regards to accuracy and precision.

Good reproducibilities were obtained upon application of the proposed methods to different blind experiments of pure samples of nafcillin sodium; the mean accuracies were 100.1 ± 0.49 and 100.3 ± 0.88 by Fe^{3+}/*o*-phen and *p*-CA methods respectively (Table 2).

Pharmaceutical Applications:

In order to evaluate the analytical usefulness of the proposed methods, they were applied to the determination of nafcillin sodium in tablets. The results were listed in Table 2 and agreed well with the reference method [24]. The results showed excellent recoveries were obtained. To ovoid the effect of the matrix interference by method 2, i. e. reduction of the absorbance signal by other sample components in tablets of nafcillin sodium, the method of standard additions was constructed to overcome these interferences.

Biological fluids applications:

The high sensitivity attained by the proposed method 1 allowed the determination of nafcillin sodium in biological fluids. The drug can be directly analysed in urine without any pretreatment. However, for plasma only a deproteination process was carried out using acetonitrile, as a sample pretreatment; an extraction procedure was not necessary. Results obtained are listed in Table 3, where the recoveries of the studied drug are 99.6 and 94.5 from urine and plasma respectively.

Table 1: Performance data for spectrophotometric determination of nafcillin sodium with the mentioned reagents

Parameter	Proposed methods using	
	Fe^{3+} / *o*-phen	*p*-CA
λ_{max} (nm)	510	530
Linearity range, (μg ml^{-1})	1.25-15	15-200
	(n = 9)	(n = 10)
Regression equation:		
Intercept (a)	-0.049	-0.032
S_a	0.003	0.002
Slope (b)	0.130	0.002
S_b	0.00	0.00
Correlation coefficient (r)	0.9999	0.9998
LOD (μg ml^{-1})	0.12	3.61
LOQ (μg ml^{-1})	0.39	12.11
% RSD (n=10)	0.94	1.9

S_a: Standard deviation of intercept.
S_b: Standard deviation of slope.

Table 2: Determination of nafcillin sodium in pure form and tablets by the proposed and reference methods.

Preparation	Fe^{3+} / *o*-phen Method	*p*-CA Method	Reference Method [24]
Pure nafcillin sodium			
% Found ± S. D.	100.1 ± 0.49	100.3 ± 0.88	100.9 ± 0.58
	n = 9		
t - value	0.78 (2.145)	0.39 (2.131)	
F - value	1.42 (3.58)	2.26 (3.37)	
Nafcillin sodium tablets *			
(250 mg / tablet)			
% Recovery ± S. D.	100.1 ± 0.49	100.4 ± 0.75	100.26 ± 0.46
t - value	0.25 (2.365)	0.198 (2.776)	
F - value	1.14 (5.79)	2.66 (19)	

* Prepared tablets containing the drug and the tablet excipients: lactose (350 mg), starch (30 mg), magnesium stearate (6.5 mg) and talc (31.5 mg) per tablet [26].
Figures in parentheses are the theoretical t and F values at p = 0.05

Table 3: Determination of nafcillin sodium in spiked urine and plasma using the (Fe^{3+} / *o*-phen) reagent.

	% Recovery	
	Urine	Plasma
	100.3	92.0
	100.8	96.0
	99.0	95.3
	98.3	94.7
Mean ± S. D.	99.6 ± 1.15	94.5 ± 1.75

Conclusion:

In this work, both ferric - phenanthroline and p-chloranilic acid were found to be efficient chromogenic reagents for the spectrophotometric determination of nafcillin sodium. The two proposed methods are simple and have also the advantages of high accuracy and precision with low detection limits compared with the published spectrophotometric methods which involve heating the drug solution with ammonium vanadate [24] or ammonium molybdate [25] in acidic medium. The proposed methods are also easier and cheaper to perform than HPLC separations and do not require expensive reagents. These advantages coupled with acceptable precision make these methods suitable for routine quality control. Method - 1 is more sensitive than method - 2 and can be applied to biological fluids.

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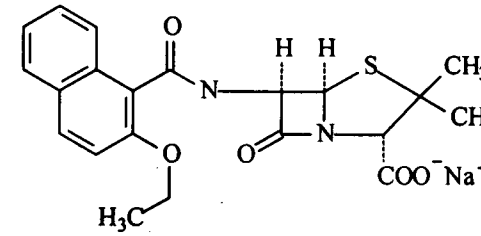

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