Spectrophotometric determination of Procaine penicillin in pure and pharmaceutical formulations using metol

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Abstract:

A simple, accurate and sensitive spectrophotometric method for the determination of Procaine penicillin (PP) is described. The method is based on charge-transfer reaction of PP with metol (N-methyl-p-hydroxy aniline) in the presence of ferric sulphate to form a purple-water soluble complex ,which is stable and has a maximum absorption at 510 nm .A graph of absorbance versus concentration shows that Beer's low is obeyed over the concentration range of 3-80 µg /ml of PP (i.e.,3-80 ppm) with a molar absorbativity of 4.945×10^3 $L.mol^{-1}.cm^{-1}$,Sandell sensitivity of 0.1190 µg cm^{-2} ,a relative error of (-1.57)-2.79 % and a standard deviation of less than 0.59 depending on the concentration of PP.The optimum conditions for full colour development are described and the proposed method was probably applied satisfactorily to pharmaceutical injections containing PP.

Key words: Procaine penicillin, Spectrophotometry, metol, charge-transfer reaction

Introduction:

Antibiotics that contain the β-Lactam (a four-member cyclic amide) ring structure constitute the dominant class of agents currently employed for the chemotherapy of bacterial infections. The first antibiotic, which was used in therapy, penicillin G (or benzyl penicillin), remains the agent of choice for the treatment of infections caused by most species of gram-positive bacteria.

The first widely used amine salt of penicillin G was made with procaine. A large number of preparations for injections of PP are commercially available [1]. Various methods have been reported for the determination of PP in pharmaceutical preparations .These methods are spectrophotometric[2],high performance liquid chromatography [3], liquid chromatography-mass spectrometry [4] and mercurimetric titration [5].Charge-transfer reactions were used for the determination of many drugs such as catecholamine [6], diclofenac sodium [7] and benzocaine[8]. Aim of research

The objective of the present investigation reported in this paper was to evaluate a spectrophotometric method for the determination of PP based on the charge –transfer reaction of this drug with metol and in the presence of ferric sulphate as oxidizing agent.

Experimental:

1 Apparatus

All spectra and absorbance measurements were carried out on a Shimadzu UV-visible 260 digital double beam recording spectrophotometer using 1-cm silica cells.

2 Reagents:

All chemicals used were of analytical reagent grade and pure Procaine penicillin drug sample was kindly provided from state company for Drug Industries and Medical Appliance,SDI,Samara.Iraq .Dosage forms were obtained from commercial sources.

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Procaine penicillin stock solution (1000 µg ml^{-1})

A 0.1000 gm amount of PP was dissolved in distilled water and the solution was made up to volume of 100 ml in volumetric flask with the same solvent . To obtain PP working solution (500 µg ml-1) a 50 ml volume of the stock solution was transferred into a 100 ml volumetric flask and made up to the mark with distilled water.

Ferric sulphate solution $Fe_2(SO_4)_3.9H_2O$ (1×10^{-2} M)

A 0.5617 gm of ferric sulphate was dissolved in distilled water and made up to 100 ml volumetric flask with distilled water.

Metol reagent solution (1×10^{-2} M)

A 0.1230 gm of pure metol was dissolved in distilled water and made up to 100 ml volumetric flask with the same solvent.

3 Procedure of pure drug

Into a series of 25 ml calibrated flask, transfer increasing volumes of PP working solution (500 µg.ml-1) to cover the range of the calibration graph 75-2000 µg of PP in a final volume of 25 ml .Add 2 ml of 1×10-2 M metol and 2 ml of 1×10-2 M of ferric sulphate solution and shake well .Dilute the solution to the mark with distilled water .Shake and allow the reaction mixture to stand for 20 min at room temperature. Measured the absorbance at 521 nm against a reagent blank prepared in the same way but containing no PP. The color of the formed product is stable for 120 min.For optimizations of conditions and in all subsequent experiments, a solution of 1000 µg of PP in a final volume of 25 ml (i.e.40 ppm) was used.

4 Procedure of Procaine penicillin injections (400 mg) (300 mg Procaine penicillin+ 100 mg benzyl penicillin)

An accurately weighed portion from mixed three vials powder , equivalent to about 0.0250 gm of PP , was transferred to a 100 ml volumetric flask and was dissolved and completed to the mark with distilled water . The flask with its content was shaked well. A sample of 500 µg of PP in a final volume of 25 ml was taken and the measurement was carried out using standard additions method.

Results and discussion

1 Absorption spectra

When a very dilute aqueous solution of PP was mixed with metol reagent in neutral aqueous medium and oxidized with ferric sulphate, an intense purple colour forms immediately, which became stable after 20 min. The purple product has a maximum absorption at 510 nm .Fig.1 shows the spectra of the purple product formed and the reagent blank, the maximum absorption at 510 nm was used in all subsequent experiments.

2 Study of the optimum reaction conditions

The effects of various parameters on the absorption intensity of the formed product were studied and the reaction conditions were optimized.

2.1 Effect of oxidant concentration

The product formation reached maximum with about 2 ml of 1×10^{-2} M of ferric sulphate solution and remained at this maximum when 0.3-4.0 ml of the oxidant concentration was added to PP,2 ml volume of oxidizing agent solution was therefore used in the procedure since it give high sensitivity, minimum blank value and ensure a quantitative determination at the upper limit of calibration graph(Table1).

2.2 Effect of reagent concentration

When various concentration of metol solution were added to a fixed amount of PP solution,2 ml of 1×10^{-2} M solution was

found enough to develop the color to its full intensity and give a minimum blank value and was considered to be optimum for the concentration range of 75-2000 µg/25 ml of PP(Table2).

2.3 Effect of order of addition

To obtain optimum results the order of addition of reagents should be followed as given under the procedure (PP+metol+ $Fe_2(SO_4)_3.9H_2O$), otherwise a loss in color intensity and stability were observed.

2.4 Effect of temperature

The effect of temperature on the color intensity of the product was studied .In practice, the same absorbance was obtained when the color was developed at room temperature (25°C),but when the calibrated flask was placed in an ice-bath (0°C) or in a water-bath at (45°C) a loss in color intensity and stability were observed ,It is therefore recommended that the color reaction should be carried out at room temperature (25°C) .

2.5 Effect of reaction time

The color intensity reached a maximum after PP solution had been reacted with metol and ferric sulphate for 20 min, therefore 20 min development time was selected as optimum in the general procedure .The colour obtained was stable for 120 min.

3 Accuracy and precision

To determine the accuracy and precision of the method, PP was determined at three different concentration .The results shown in Table (3); indicate that a satisfactory precision and accuracy could obtained with the proposed method.

4 Calibration graph

Employing the conditions described under procedure , a linear calibration graph Fig.2 for PP was obtained , which shows that Beer's law obeyed over the concentration range of 75-2000 µg/25 ml or 3-80 ppm with a correlation coefficient of 0.9998 .

The conditional molar absorptivity of the product formed with PP was found to be 4.945×10^3 $L.mol^{-1}.cm^{-1}$ with reference to the PP and sandell sensitivity was 0.1190 $\mu g.cm^{-2}$.

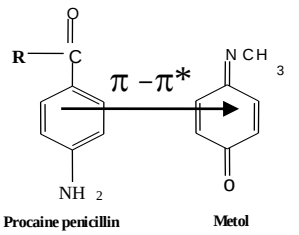
5 Analytical application

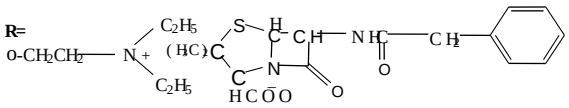
Three types of injections containing PP have been analyzed .It was found that, when the proposed method was applied to the determination of PP in injections , the % recovery was around 122% ,this might be due to the interaction of the benzyl penicillin that present with PP injections.

Therefore, a standard additions method is applied (Fig.3) which I nvolves adding increment volumes (0-6 ml) of a standard solution of 250 $\mu g.ml^{-1}$ of PP to a fixed volume sample (2 ml of 250 $\mu g.ml^{-1}$ of pharmaceutical preparations) and employing the conditions described under procedure .They gave a good accuracy and precision (Table 4).The proposed method was compared successfully with the British pharmacopeia's standard method (Table 5).

Structure of the product

The stoichiometry of the formed charge- transfer complex between each PP and metol was investigated under the recommended optimum conditions and applying molar ratio method .The results obtained Fig.4 show that a (1:1) charge–transfer complex was formed between PP and metol reagent at 510 nm ,therefore, the formation of product probably occurs as follows.





The purple colored product can be described as charge-transfer complex formation that takes place through a π-π* transition between PP and metol [6]. The stability constant of the product in water under the described experimental conditions was 1.36×10^4 $L.mol^{-1}$[9] .

Conclusion:

A simple, accurate and sensitive spectrophotometric method has been developed for the determination of trace amount of PP in aqueous solution based on charge-transfer complex with metol reagent and ferric sulphate .

The proposed method does not require temperature control or solvent extraction step, the method was applied successfully to pharmaceutical injections. A stable-soluble purple color complex was formed which can be measured at 521 nm . The method does not require temperature control or solvent extraction step and can be applied successfully to pharmaceutical injections containing PP.

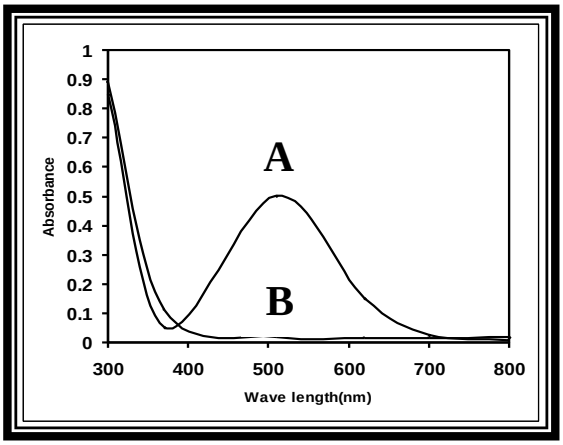
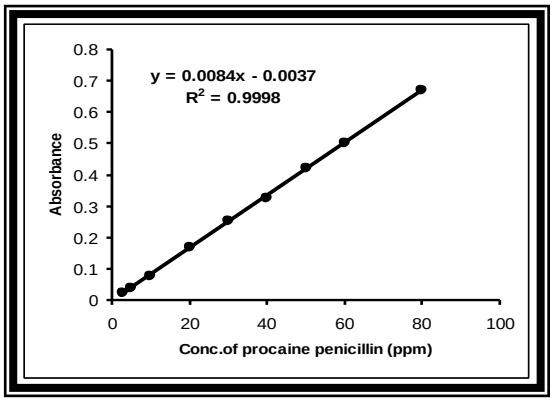
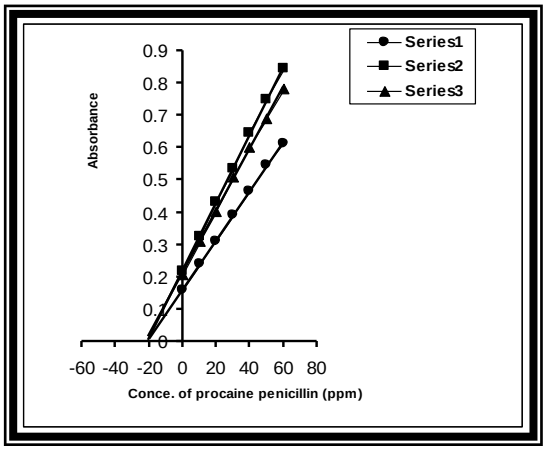


Fig. (1) Absorption spectra of A (60 µg ml^{-1}) of PP treated as described under procedure and measured against blank and B the reagent blank measured against distilled water.



Fig(2) :Calibration graph of Procaine penicillin



Fig(3): Standard additions method for determination of PP in pharmaceutical injections (Series1:PP France-panpharm,Series2:PP Ajanta-India, Series3: PP Alimbca-India).

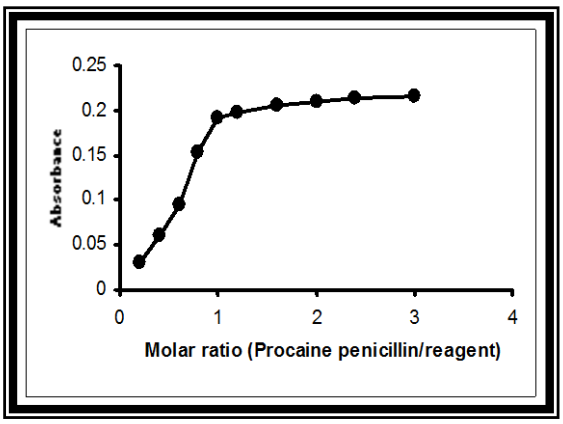


Fig (4): Molar ratio of Procaine penicillin to reagent for the product formed.

The concentrations of both the sample and reagent are 8.49×10^{-4}M.

Table(1):Effect of the volume of oxidant (1×10^{-2} M) on the absorption of the formed product.

Volume of $(Fe_2(SO_4)_3.9H_2O$(ml)	Absorbance
0.3	0.180
0.5	0.236
1.0	0.284
1.5	0.310
2.0	0.320
2.5	0.312
3.0	0.311
3.5	0.309
3.5	0.306

Table(2):Effect of the volume of reagent (1×10^{-2} M) on the absorption of the formed product.

Volume of metol (ml)	Absorbance
0.3	0.190
0.5	0.282
1.0	0.313
1.5	0.316
2.0	0.323
2.5	0.311
3.0	0.309
5.0	0.289

Table (3): Accuracy and precision of the proposed method.

Amount of PP ($\mu g.ml^{-1}$)		Recovery%*	R.S.D %*
Present	Found		
40.00	39.36	98.42	0.216
60.00	60.08	100.13	0.282
80.00	80.32	100.40	0.227

*Average of four determinations.

Table(4): Application of the proposed method for the determination of PP in pharmaceutical injections using standard addition method.

PP injection Sample	Wt of PP (mg)	PP ($\mu g.ml^{-1}$)		R.S.D %*	Rec. %*
		Taken	Found		
Procaine penicillin France-Panpharm	400	20.00	20.00	1.157	100.00
Procaine penicillin Alimbca-India	400	20.00	19.90	0.905	99.50
Procaine penicillin Ajanta-India	400	20.00	20.20	0.632	101.00

* Average of five determinations.

Table (5): Comparison of the proposed method with standard method to determination of PP in pharmaceutical injections.

Procaine penicillin injection	Recovery%*	
	Proposed method	Standard method**
Pure Procaine penicillin	100.43	101.58
Procaine penicillin France-Panpharm	100.00	101.50
Procaine penicillin Alimbca-India	99.50	99.82
Procaine penicillin Ajanta-India	101.00	100.25

* Average of five determinations.

** British Pharmacopoeia standard method[10].

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القدير الطيفي للبروكائين بنيسيلين في الاشكال الصيدلانية والنقية بأستخدام الميتول

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الخلاصة:

يتضمن البحث تطوير طريقة طيفية سريعة للتقدير الكمي للمقادير الضئيلة من مستحضر البروكائين بنيسيلين في المحاليل المائية باستخدام الطريقة الطيفية . تعتمد الطريقة على تفاعل انتقال الشحنة بين البروكائين بنيسيلين وكاشف الميتول بوجود العامل المؤكسد كبريتات الحديديك حيث يتكون ناتج ارجواني مستقر وذائب في الماء اعطى اعلى امتصاص عند طول موجي 510 نانومتر .يشير الرسم البياني للامتصاص مقابل التركيزبان قانون بير ينطبق ضمن مدى التركيز 3-80 مايكروغرام/مل من البروكائين بنيسيلين (اي ما يكافئ 3-80 جزء بالمليون) وكانت قيمة الامتصاصية المولارية مساوية الى 4.945×10^3 لتر.مول$^{-1}$.سم$^{-1}$ وقيمة حساسية ساندل 0.1190 مايكروغرام .سم$^{-2}$ مع خطأ نسبي (-1.57)-2.79 % وانحراف قياسي نسبي اقل من 0.59 أعتمادا على مستوى التركيز المراد تقديره.تمت دراسة الظروف المثلى للتفاعل وتطبيق الطريقة على الحقن الحاوية على البروكائين بنيسيليسن.