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SELECTIVE SPECTROPHOTOMETRIC METHODS FOR THE ANALYSIS OF D-PENICILLAMINE BULK DRUG AND CAPSULES IN THE PRESENCE OF SOME PENICILLINS AND THEIR DEGRADATION PRODUCTS

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تم استنباط ثلاث طرق بسيطة وسريعة وحساسة للتعيين الطيفى للبنسلامين فى وجود بعض البنسلينات ونواتج تحللها.

تعتمد هذه الطرق الثلاث على اكسدة البنسلامين بأيون الحديديك فى الوسط المائى وقياس متراكب الحديدوز مع كل من ٢،٢-بايبيريديل وحديدى سيانيد البوتاسيوم وكذلك ١،١٠-فينانثرولين عند درجة امتصاص قصوى ٠,٥٢٣ ، ٠,٦٩٠ ، ٠,٥١٠ نانوميتر على الترتيب.

وقد تم دراسة المتغيرات المؤثرة على التفاعل. وقد وجد من التحليل الاحصائى أن التفاعلات تتبع قانون بيير عند تركيزات تتراوح بين ١-١٠ ميكروجرام لكل ملليليتر من البنسيلامين.

ولا يوجد تداخل من بنزيل البنسلين وبنزاثيد بنسلين وبروكايين بنسلين والبنسلين اللامائى وصوديوم بنسلين وحامض بنزايل بنسلويك وحامض ٦-امينوبنسلانيك وبنسلامين ثنائى الكبرتيد بالاضافة الى الاضافات الصيدلية المعتادة الموجودة فى الكبسولات.

وقد تبين أن الطرق المقترحة حساسة للغاية كما أنه أمكن تطبيقها فى تحليل الارتامين كبسولات بنسبة استرجاع تتراوح بين ٩٩,٥ - ١٠٠,٩٪.

Three simple, rapid and sensitive spectrophotometric methods for the assay of D-penicillamine in the presence of some penicillin preparations as well as common penicillin degradation products were carried out. These methods are based on the oxidation of penicillamine with ferric ion in aqueous solution. The indirect quantitation of the produced ferrous ions was carried out at 523, 690 and 510 nm for ferro-bipyridyl, ferro-ferricyanide and ferro-1,10-phenanthroline complexes respectively. All variables were studied to optimize the reaction conditions. Regression analysis of Beer's plot showed good correlation in a general concentration range of 1-10 µg D-penicillamine/ml. No interference could be observed from the presence of many structurally relevant compounds. In addition, common pharmaceutical adjuvants present in capsules did not interfere. The validity of the proposed methods was tested by analyzing artamine capsules. Recoveries were 99.5 - 100.9%.

INTRODUCTION

Although penicillamine is not an antibiotic, D-penicillamine is considered to be a certifiable drug either in bulk or formulations, because it is a derivative of penicillin. It is used in medicine as a metal chelating agent and possibly as antirheumatic. Numerous analytical methods have been developed for the quantitative analysis of D-penicillamine[1]. Extensive studies on the chelating properties of D-penicillamine towards variety of metals have been reported[2-6], but analytical procedures for its quantitative determination have been developed more for biological[7-13] than for pharmaceutical systems[14-18]. The official USP method[19] involves the use of highly toxic mercuric acetate. An alternative investigations such as nonaqueous

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titrations and hydroxylamine colorimetric assay have been also reported[20].

Due to the absence of chromophores and/or auxochromes in the penicillamine molecule, native uv spectrophotometry can not be adopted for its analysis. Therefore, it is highly desirable to find colorimetric procedures suitable for routine analysis. In addition, the previously reported procedures are sophisticated and time consuming, thus unsuitable for the analysis of large number of samples. Moreover, no analytical procedures are available to the author's knowledge concerning the analysis of D-penicillamine in the presence of other related penicillins and their degradation products.

The aim of this investigation is to develop simple chemical procedures for the determination of D-penicillamine in pure, capsules as well as in intact and degraded penicillin preparations. The proposed methods are based on the oxidation of its mercapto group with iron (III) in the presence of 2,2'-bipyridyl, potassium ferricyanide or 1,10-phenanthroline. The iron (II) formed was quantitatively and rapidly converted to the corresponding iron (II)-complexes which can be measured spectrophotometrically at 523, 690 and 510 nm for 2,2'-bipyridyl, potassium ferricyanide and 1,10-phenanthroline complexes respectively.

EXPERIMENTAL

Apparatus:

A uvidec-320 (Jasco, Japan) and a Perkin Elmer, model Lambda 3 B UV/Vis (USA) spectrophotometers with two matched 1 cm quartz cells were used.

Reagents:

2,2'-Bipyridyl (Prolabo, France), potassium ferricyanide (Riedel De Haen, France), 1,10-phenanthroline (E. Merck, Germany) and ferric sulphate (Riedel De Haen, France), 0.2% W/V solutions were separately prepared in distilled water.

Sodium fluoride, (Arabic Lab. Equipment Co., Egypt) 1.0% solution was prepared in distilled water.

D-penicillamine, benzylpenicillin, benzathine penicillin, procaine penicillin, anhydrous ampicillin, sodium ampicillin, penicillic acid, 6-aminopenicillanic acid and penicillamine disulfide authentic samples were obtained from Sigma Co., Germany. Benzylpenicilloic acid was prepared by a standard method[21]. Artamine capsules, containing 250 mg D-penicillamine (Biochemia Austria) was obtained from local market.

All reagents were of analytical-reagent grade and were used without further purifications.

Standard solution:

An accurately weighed amount of D-penicillamine was dissolved in a known volume of distilled water. This stock solution was diluted to obtain working solutions containing 10-100 μg/ml of D-penicillamine.

Capsules:

The contents of 20 capsules were mixed and an amount equivalent to 10 mg of the drug was accurately weighed, dissolved in water and diluted to 100 ml. After well mixing, the solution was directly used for the assay.

Interferences:

Fifty milligrams of D-penicillamine was mixed with each of the penicillin preparations and the degradation products presented in Table 4. The mixed contents were transferred into a 100-ml calibrated flask, shaken with 50 ml of distilled water for about 15 min, completed to the mark with distilled water. The resulting solution was filtered, rejecting the first portion of the filtrate.

General Procedure:

1.0 ml of the standard or capsule solution containing 100 μg D-penicillamine was transferred into a 10-ml volumetric flask, and after the addition of 1.0 ml of 0.2% ferric sulphate solution and 1.0 ml of complexing agents (2,2'-bipyridyl or potassium ferricyanide or 1,10-phenanthroline reagents), the mixture was shaken and allowed to stand for 15 min. Then 1.0 ml of 1.0% sodium fluoride solution

was added, completed to 10 ml with distilled water. The absorbance was measured at 523, 690 and 510 nm for 2,2'-bipyridyl, potassium ferricyanide and 1,10-phenanthroline respectively against a reagent blank prepared side by side.

RESULTS AND DISCUSSION

The proposed methods are based on the ability of the mercapto-group to reduce iron (III) to iron (II), which is rapidly converted to the corresponding stable colored reagent-iron (II) complex according to the following reactions:

$$2\ Fe^{3+} + 2\ R\text{-}SH = 2\ Fe^{2+} + R\text{-}S\text{-}S\text{-}R + 2\ H^{+}$$

$$Fe^{2+} + 3(2,2'\text{-Bipyridyl}) = [Fe(2,2'\text{-Bipyridyl})_3]^{2+}$$

$$Fe^{2+} + [Fe(CN)_6]^{3-} + K^{+} = KFe[Fe(CN)_6]$$

$$Fe^{2+} + 3(O\text{-Phenan.}) = [Fe(O\text{-Phenan.})_3]^{2+}$$

The produced colored products exhibit absorption maxima at 523, 690 and 510 nm for ferro-bipyridyl, ferro-ferricyanide and ferro-1,10-phenanthroline respectively, Fig. 1, whereas the reagents and drugs give no absorption at these maxima.

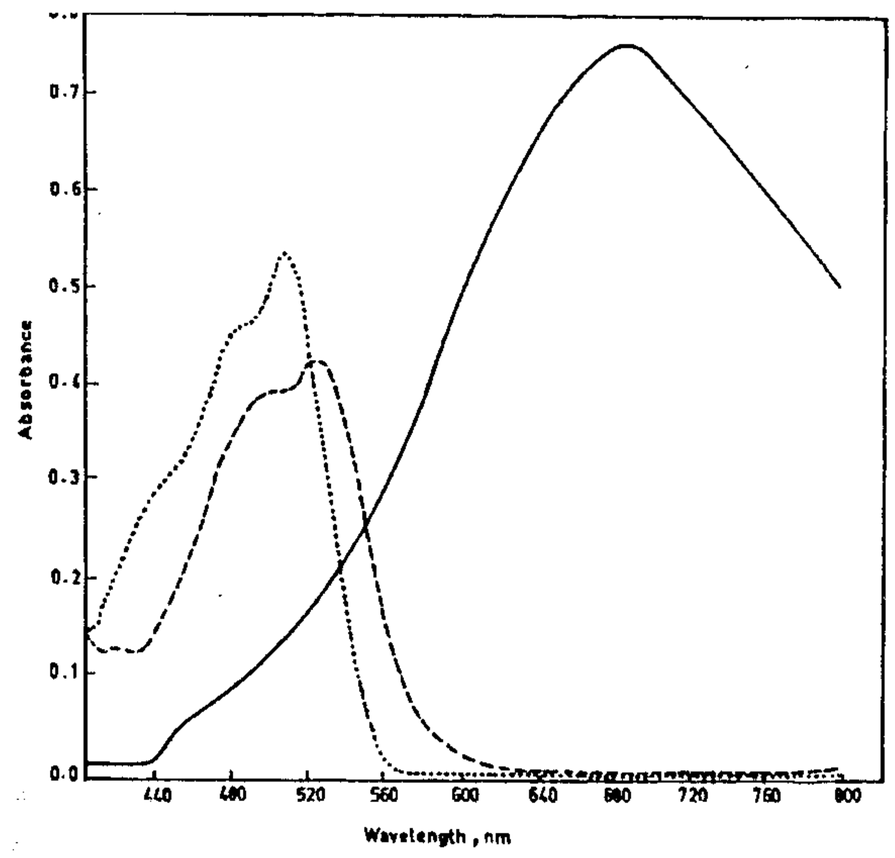

Fig. 1: Absorption Spectra of [Fe^{2+} - bibyridyl] -----, [Fe^{2+} - Ferricyanide] ———— and [Fe^{2+} - phenanthroline] ..... complexes with 8 μg D-penicillamine/ml.

The conditions under which the reaction of D-penicillamine with iron (III) in the presence of 2,2'-bipyridyl, potassium ferricyanide or 1,10-phenanthroline fulfills the necessary analytical requirements were investigated.

Table 1: Solvent Effect on the Wavelength of maximum Absorption (using 10 μg D-penicillamine/ml).

Solvent added	2,2'-Bipyridyl		Potassium Fericyanide		1,10-Phenanthroline		DEC**
	A*	λ_{max}	A*	λ_{max}	A*	λ_{max}	
Water	0.533	523	0.940	690	0.676	510	80.2
DMSO	0.427	523	0.617	520	0.670	515	47.0
Methanol	0.530	520	0.930	700	0.670	507	32.6
Ethanol	0.400	520	0.617	650	0.673	508	24.3
Acetone	0.463	519	0.600	630	0.675	510	20.7
1-Propanol	0.455	520	0.731	640	0.678	510	21.8
2-Propanol	0.415	520	0.630	632	0.669	510	19.9
1,4-Dioxane	0.295	523	0.460	630	0.665	510	2.2

* Mean of three determinations.

** Reference 23.

Dilution of the developed colored product by different solvents brings about some changes in the position of λ_{max}. The absorption intensity was highly affected on using 2,2'-bipyridyl and potassium ferricyanide and little affected with 1,10-phenanthroline, Table 1. It was found that, within water, methanol and ethanol the absorbance readings of the reaction products increase with increasing the dielectric constant. This could be explained on the basis of the increase in the polarity of the excited state of the resulting complexes with increasing the dielectric constant of the solvent. Water and methanol were found to be the best. Therefore, Water was used throughout this work as it is the cheapest one.

The absorbance readings of the resulting reaction products were found to increase with increasing the volume of iron (III) solution, 1.0 ml of 0.2% ferric sulphate solution was found optimal. It was found that, the reaction between the drug and ferric ion goes rapidly at the first 15 min (at 20-25°C) and then proceeds very slowly. Figure 2 revealed that up to 96% of the color developed in 1 hr has been completed within the first 15 min. Only slight increase (4%) was observed in the next 45 min. This means that the reaction is kinetically slow after the first 15 min. It was reported that mono-mercapto-compounds are oxidized by iron (III) to the corresponding disulfide derivatives and the stoichiometry of this reaction was found to be 1:1[22]. On the other hand penicillaminic acid has been reported to be the product of oxidation of D-penicillamine upon using bromine water[1]. Therefore, the use of 1.0 ml of 1.0% sodium fluoride solution is necessary after 15 min for quenching the reaction in order to obtain stable absorbance readings. One milliliter volume of 0.2% (w/v) of 2,2'-bipyridyl, potassium ferricyanide or 1,10-phenanthroline was found suitable for the assay procedure.

A linearity study of the three approaches were under-taken by constructing calibration curves of absorbance versus concentration in the range of 1-10 μg/ml. The parameters and correlation coefficients of the calibration plots, expressed as regression lines of the form Y = a + bX are listed in Table 2. As it can be seen from the equations obtained, the lines pass very close to the origin and the slopes remain high with good correlation coefficients. To examine the precision of the proposed procedures, eight replicate determinations were performed using 10 μg D-penicillamine/ml (Table 3). The percentage coefficients of variation ranged between 0.530 and 0.798.

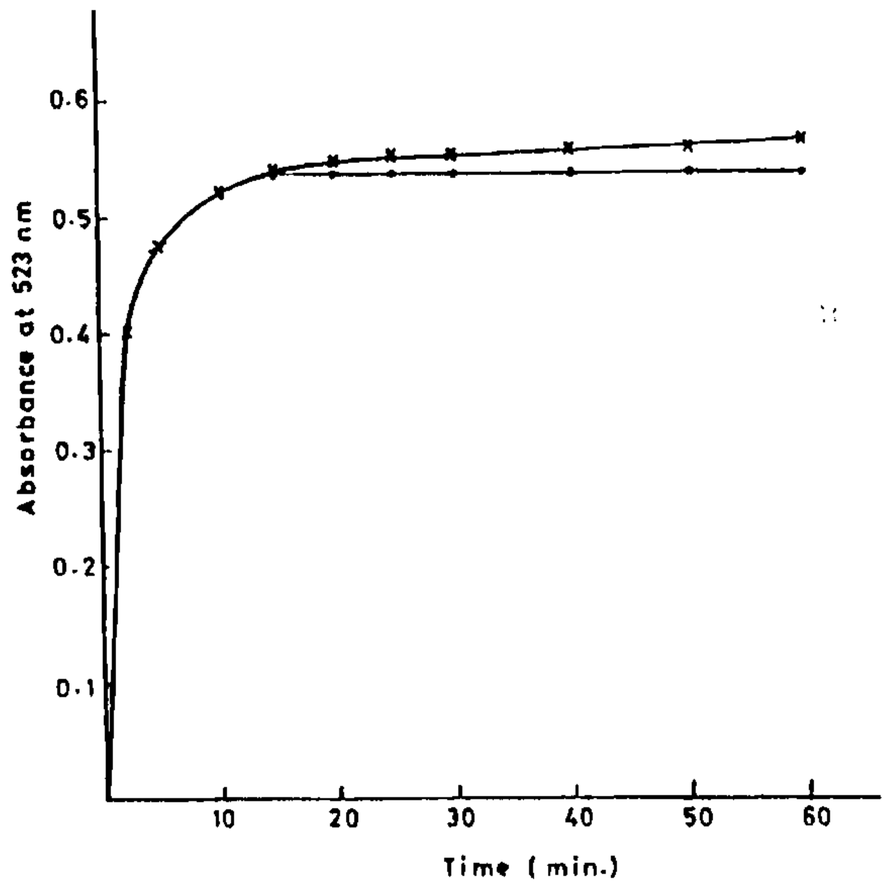

Fig. 2: Effect of time on the color development at 523 nm with 10 μg D-penicillamine/ml (x-x-x) and (•-•-•) with sodium fluoride.

As penicillamine is a major degradation product for β-lactam antibiotics, obtained through an acid hydrolysis, possible interference from penicillin molecules and/or their structurally related degradation products exist[1] which might be originally present in penicillin samples or produced during hydrolysis procedure. Therefore analysis of D-penicillamine in the presence of some penicillin preparations and their related degradation products by the proposed procedures was carried out. No interference could be observed from the presence of the substances listed in Table 4, specially from penicilloic acid which is expected to be oxidized with ferric ions as it is removed by filtration.

The proposed procedures were applied to the quantitative determination of artamine capsules containing 250 mg of D-penicillamine. Three different lots were analyzed to compare the proposed procedures. The statistical data for capsule determination presented in Table 5, show that the methods are precise with an average % CV of 0.502-0.654.

In conclusion, the proposed procedures are equally recommended and allow simple, reproducible, sensitive and selective determination of D-penicillamine in bulk drug, and capsules with good accuracy (99.5 - 100.9% recovery) and remarkable precision. In addition, the proposed procedures suggest easily available reagents for the determination of penicillamine in penicillin preparations containing other structurally related degradation products. On the other hand, these procedures are comparatively superior in terms of cost, convenience and sample handling.

Table 2: Comparative Statistical Evaluation for D-Penicillamine Analysis by the proposed procedures.

Method	λ_{max} nm	ϵ, $x10^{-4}$	Linear range μg/ml	a*	b*	r*
Bipyridyl	523	0.79	1 - 10	-0.009	0.0539	0.9999
Ferricyanide	690	1.43	1 - 10	0.064	0.0891	0.9981
Phenanthroline	510	1.00	1 - 10	0.019	0.0691	0.9994

ϵ = molar absorptivity ($l.mol^{-1}cm^{-1}$)
b = slope
* in all cases n = 6.
a = intercept.
r = correlation coefficient.

Table 3: Replicate Analysis of 10 μg/ml D-penicillamine Standard Solutions by the Three Different Approaches.

Replicates	2,2'-Bipyridyl (523)	Absorbance at λ_{max} (nm)	
		Potassium Ferricyanide (690)	1,10-Phenanthroline (510)
1	0.530	0.950	0.679
2	0.536	0.960	0.682
3	0.528	0.953	0.669
4	0.527	0.948	0.665
5	0.532	0.955	0.670
6	0.532	0.952	0.672
7	0.529	0.947	0.675
8	0.528	0.962	0.678
Mean	0.530	0.953	0.674
± SD	$2.96x10^{-3}$	$5.05x10^{-3}$	$5.75x10^{-3}$
CV, %	0.560	0.530	0.850

Table 4: Analysis of D-penicillamine in the Presence of Other Penicillins or Their Degradation Products.

Interfering Compound*	% Recovery**, ± SD		
	2,2'-Bipyridyl	Potassium ferricyanide	1,10-Phen-anthroline
Benzylpenicillin[1]	100.75 ± 0.93	99.06 ± 1.29	100.74 ± 1.12
Penicilloic acid[2]	100.57 ± 0.71	99.48 ± 1.38	100.15 ± 0.54
Penicillic acid[3]	99.43 ± 0.67	99.48 ± 0.55	99.70 ± 0.86
6-APA[2]	101.89 ± 0.78	100.52 ± 0.83	101.04 ± 1.22
Benzathine pen.[1]	100.57 ± 0.53	100.31 ± 0.77	100.45 ± 0.85
Procaine pen.[1]	100.19 ± 1.22	99.69 ± 0.48	99.70 ± 0.90
Anhyd. Ampicilllin[1]	99.06 ± 0.90	101.15 ± 1.12	101.19 ± 0.75
Sod. Ampicillin[1]	101.89 ± 0.83	101.57 ± 0.85	100.89 ± 1.30
Pen. Disulphide[1]	99.62 ± 1.07	99.48 ± 1.20	99.70 ± 0.92
Mean Recovery %	100.44	100.08	100.40
±SD	± 0.94	± 0.81	± 0.57

* Added in mg per 50 mg of D-penicillamine.
** Mean of three determinations.
1 = 50 mg, 2 = 25 mg, 3 = 10 mg.

Table 5: Comparison of D-penicillamine Determination in Artamine Capsules (250 mg) by Applying The Three Different Procedures in Comparison With The Official USP XXII Method.

Procedure	Lot	Av %	± SD	CV %	Overall CV %
2,2'-Bipyridyl	A	100.22	0.652	0.651	0.591
	B	100.00	0.531	0.531	
	C	99.89	0.326	0.326	
Potassium Ferricyanide	A	99.50	0.784	0.788	0.654
	B	100.10	0.501	0.500	
	C	100.08	0.675	0.674	
1,10-Phenanthrolline	A	100.86	0.583	0.578	0.562
	B	99.91	0.489	0.489	
	C	100.15	0.621	0.620	
USP-XXII*	A	101.27	1.205	1.190	1.195
	B	101.06	0.578	0.573	
	C	101.61	1.852	1.823	

* Reference 19.

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