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Short Communication

Analytical investigations of β-lactam antibiotics in pharmaceutical preparations — I. Spectrophotometric determination of cephalexin, cephradine, ampicillin and amoxycillin using paramolybdate anion

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Introduction

Several procedures have been described for the determination of β-lactam antibiotics, especially for the determination of Cephalexin, Cephradine, Ampicillin and Amoxycillin, either as the pure drug, in pharmaceutical preparations or in human fluids [1–7].

In the present study a new spectrophotometric method has been developed, based upon the reduction of paramolybdate anion ($Mo_7O_{24}^{6-}$) to molybdenum blue by the β-lactam antibiotics in the presence of sulphuric acid.

A considerable number of substances of pharmaceutical interest such as the tetracyclines [8–10], the sodium salts of Cefoxitin, Cephapirin, Cephalothin and Cephaloridine [11], as well as a number of reducing agents of natural origin (e.g. sugars, ascorbic acid) produce the same colour reaction under similar conditions.

The purpose of the present investigation was two-fold: namely to develop a rapid, accurate and convenient method for the assay of the title compounds in a pure form and in pharmaceutical formulations, such as injections and hard-filled capsules using instrumental techniques normally available in an industrial quality control laboratory.

Although standard methods which make use of the microbiological technique are equally sensitive, they are prone to greater sources of errors which limit their precision. Also they have the disadvantage that they generally require long periods of incubation.

Experimental

Apparatus

A Hitachi Model 100–80, double-beam ratio recording spectrophotometer equipped with 10.0 mm quartz cells was used during method development.

A Beckman Model DU-2 single-beam spectrophotometer, with 10.0 mm quartz cells was used for the application of the proposed method to quality control work.

An Ultrathermostat Model NBS (Gebrüder Haake K.G.) was used to control temperature during sample preparation and colour development.

Reagents

The following materials were used:

Cephalexin, provided by Chemische Fabrik Schweizerhall-Basel (Switzerland);

Cephradine, supplied by Gema-Liesa (Spain);

Ampicillin sodium, produced by Liessa (Spain);

Amoxycillin trihydrate, made by Gema-Liesa (Spain);

ammonium heptamolybdate [Merck, p.a. No. 1182, solution 0.01 M (= 1, 24% w/v in water)];

sulphuric acid (Fluka, puriss. p.a. No. 84720, solution 1.0 M in water);

standard solutions of the antibiotics were freshly prepared by dissolving the appropriate amount of each in water to form 10^{-2}M solutions;

methanol dried (max. 0.01% H_2O) GR. (Merck, GR., No. 6012).

Procedures

a. *General method.* One millilitre aliquots of 1.0 M H_2SO_4 and 10^{-2}M $(NH_4)_6Mo_7O_{24}$ respectively, are transferred to 20-ml test-tubes and mixed.

Suitable volumes of each of the standard antibiotic solutions are added to produce concentrations between 1.4–50 μg ml^{-1} of Cephalexin and Cephradine and 6–200 μg ml^{-1} of Ampicillin and Amoxycillin together with water to produce a final volume of 9–9.5 ml. The prepared solution is mixed by shaking and then placed for 30 min in a thermostat at 95 ± 1°C.

After completion of the heat treatment, the contents of the test tubes are cooled to room temperature, transferred carefully to a 10-ml volumetric flask and diluted to the mark with water prior to mixing.

The absorption of the solution is measured in the visible region (400–800 nm) against a blank sample which had been treated similarly. The typical absorption curves are shown in Fig. 1.

The quantity of antibiotic in the test solutions was determined using the appropriate calibration curve. In each case the calibration curves of absorbance versus concentration were straight lines passing through the origin.

b. *Procedure for injection solutions.* The method described in the above section was applied to injection solutions, such as Ampicillin sodium, without modification.

c. *Procedure for hard-filled capsules.* For capsules containing the pure antibiotic alone the general method is applied without modification. However, for capsules containing added excipients, such as lactose, starch, alginic acid, which may interfere with the determination, the antibiotic must be extracted into methanol (efficiency 100%) before assay.

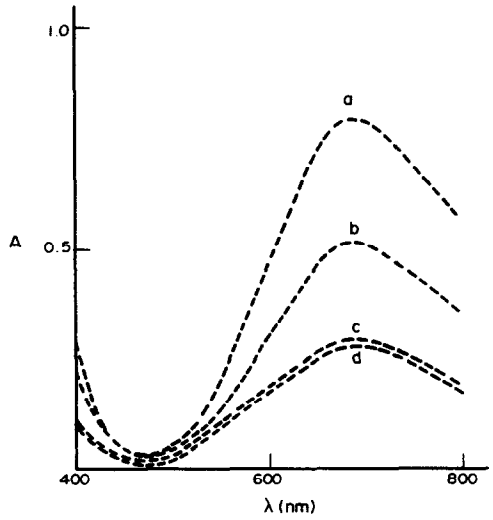

Figure 1

Thus, a suitable quantity of the mixed contents of at least 20 capsules, accurately weighed is transferred into a 50-ml volumetric flask, 30 ml methanol added, the flask shaken for 10 min and then the methanolic supernatant solution is filtered through a fast paper filter into a 100-ml volumetric flask. The extraction is repeated twice and then the 100-ml volumetric flask is made up to volume with the same solvent.

An accurately measured volume of this solution is pipetted into a 50-ml conical flask and carefully evaporated to dryness on a water bath. The residue is dissolved in about 7.0 ml of water and the assay completed as described in the first section.

Discussion and Results

When an acidified solution of paramolybdate anion is treated with a reducing agent an intense deep blue coloration is produced, due to the formation of molybdenum blue in which molybdenum is present in the 6+, 5+ or an intermediate valence state.

The formation of molybdenum blue has been used:

(i) as a qualitative analytical test for molybdenum;
(ii) for the colorimetric determination of molybdenum;
(iii) for the spectrophotometric determination of the reducing agent which is responsible for the formation of the coloration.

The present work is based on the latter, in so far as it involves the spectrophotometric determination of some β-lactam antibiotics that act as reducing agents. The results obtained by the proposed method reveal it to be both quantitative and reproducible as shown by the data in Table 1.

The method was applied to β-lactam antibiotics as the pure drug substance, in an injection solution and in hard-filled capsules.

Table 1 summarises the results obtained by means of the present method and an official method [12–13] to the determination of these β-lactam antibiotics in pure form, in laboratory-prepared formulations and in commercial pharmaceutical preparations in which the only reducing substance present was the antibiotic.

Table 1
Results obtained in a comparative study of the determination of β-lactam antibiotics in pharmaceutical preparations

No.	Test materials	Recovery* ± Standard deviation %	
		Proposed procedure	Official method†
1.	Cephalexin (I) powder	101.0 ± 0.28	101.0 ± 0.40
2.	Cephradine (II) powder	99.7 ± 0.45	99.2 ± 0.60
3.	Ampicillin (III) powder	100.8 ± 0.30	98.9 ± 0.50
4.	Amoxycillin (IV) powder	98.9 ± 0.14	99.2 ± 0.20
5.	Cephalexin Capsules‡	98.7 ± 0.45	98.1 ± 0.60
6.	Cephradine Capsules‡	99.7 ± 0.21	99.3 ± 0.36
7.	Ampicillin Capsules‡	100.9 ± 0.20	99.9 ± 0.18
8.	Amoxycillin Capsules‡	98.9 ± 0.60	99.1 ± 0.55
9.	Ceporex (I) Caps (Glaxo)	100.7 ± 0.32	101.4 ± 0.40
10.	Medalexin (I) Caps (N.P.I.)§	102.8 ± 0.40	102.0 ± 0.25
11.	Sinthecillin (I) Caps (Proel)	104.1 ± 0.10	103.8 ± 0.20
12.	Velosef (II) Caps (Squibb)	100.7 ± 0.14	102.0 ± 0.42
13.	Nipredin (II) Caps (Proel)	102.1 ± 0.10	101.1 ± 0.15
14.	Penbritin (III) Caps (Beecham)	99.9 ± 0.10	99.7 ± 0.22
15.	Pentrexyl (III) Caps (Bristol)	100.0 ± 0.23	99.8 ± 0.38
16.	Allomycin (III) Caps (Proel)	101.0 ± 0.32	99.9 ± 0.17
17.	Amoxil (IV) Caps (Beecham)	101.0 ± 0.64	101.3 ± 0.45
18.	Brilapen (IV) Caps (Bristol)	99.8 ± 0.55	100.0 ± 0.21
19.	Geymocillina (IV) Caps (Proel)	99.5 ± 0.35	100.2 ± 0.55
20.	Penbritin (III) Injs (Beecham)	102.5 ± 0.10	102.1 ± 0.34
21.	Allomycin (III) Injs (Proel)‖	104.8 ± 0.15	104.2 ± 0.15

*Average of six determinations.
†U.S. Pharmacopeia (12) (microbiological technique).
‡Laboratory-prepared, containing the antibiotic and lactose, starch and aerosil.
§National Pharmaceutical Industry of Greece.
‖The samples 20 and 21 were assayed as Ampicillin sodium.
The symbols (I), (II), (III) and (IV) correspond to Cephalexin, Cephradine, Ampicillin and Amoxycillin, respectively.

No significant differences between the results of the proposed procedure and the official method were observed.

The λ_{max} values, the corresponding molar absorptivities, the $^{1\%}_{1cm}$ A values and the colour of the measured solutions are listed in Table 2.

The products of all β-lactam antibiotics examined showed similar colour stability. In each case the developed colour attained a stable intensity for at least 1 h after the reduction of Mo(VI) to molybdenum blue.

The small differences between the λ_{max} and the corresponding colours which were observed in connection with the nature of the reducing agent is a common phenomenon which has been reported previously [14]. Thus, when Mo(VI) is reduced to molybdenum blue in acid medium (pH = 0.6–1.4) the maximum absorption varies by reducing agent in the order: ascorbic acid 800 nm, thiourea 810 nm, stannous ion 680 nm, ferrous ion 700 nm etc. The reasons for these variations of the λ_{max} is not yet fully understood.

Table 2
Spectral characteristics of the analyte solutions

β-lactam antibiotic*	λ_{max}(nm)	Molar absorptivity ($l\ mol^{-1}\ cm^{-1}$) (†)	$^{1\%}_{1cm}A$†	Colour of measured solution
Cephalexin (I)	694	9.7×10^3	279.4	Deep blue
Cephradine (II)	694	1.5×10^4	432.2	Deep blue
Ampicillin (III)	691	5.5×10^3	158.3	Blue
Amoxycillin (IV)	687	5.4×10^3	155.5	Emerald blue

* All the antibiotics are in pure form. The results after extraction are the same.
† Average of six determinations.

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