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Short Communication

Analytical investigations of β-lactam antibiotics in pharmaceutical preparations — III. Spectrophotometric determination of some cephalosporins using paramolybdate anion

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Introduction

In a previous paper [1] it was demonstrated that the paramolybdate anion in an acid environment is a sufficiently sensitive reagent for the spectrophotometric determination of some β-lactam antibiotics by formation of a blue solution. Abdel-Khalek *et al.* [2], Morelli *et al.* [3, 4] and Sultan [5] proposed similar methods with the same reagent for the colorimetric assay of some cephalosporins and tetracyclines.

The purpose of this study was to devise a modification of the above methods based on changes in some experimental conditions (acidity, concentration of paramolybdate anion, temperature and time of heating) so that the blue colour is stable for a longer period, i.e. for at least 4–6 h; the λ_{max} is displaced, under the studied conditions, to 810 nm.

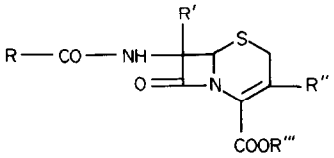
The described method gave adequate sensitivity (as the values Sandell's sensitivity indicate), good precision and satisfactory reproducibility of analytical results, and has been applied favourably to the determination of five cephalosporins either in pure form or in injections. Two other advantages for the proposed procedure are simplicity and speed. The cephalosporins analysed were: cefaclor (**I**), cefazolin sodium (**II**), cefotaxime sodium (**III**), cefoxitin sodium (**IV**) and cefamandole nafate (**V**); these antibiotics belong to the first (**II**), second (**I**, **IV**, **V**) and third (**III**) generation of cephalosporins. The chemical structures of these cephalosporins are shown in Table 1.

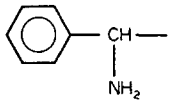
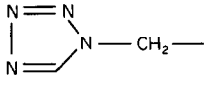
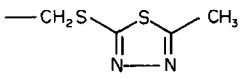
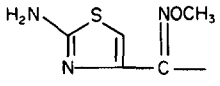
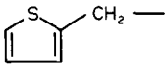
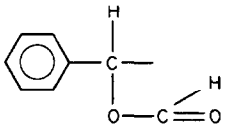
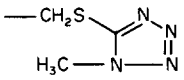
Experimental

Apparatus

A Perkin–Elmer 124 double-beam spectrophotometer and a Perkin–Elmer 139 single-

Table 1
Chemical structures of cephalosporins



Cephalosporin	R	R′	R″	R‴
I		—H	—Cl	H
II		—H		Na
III		—H	—CH_2OCOCH_3	Na
IV		—OCH_3	—CH_2OCONH_2	Na
V		—H		Na

beam spectrophotometer were used for the absorbance measurements during the application of the proposed method under industrial conditions.

A Hitachi Model 100-80 double-beam ratio recording spectrophotometric system, with 10.0-mm quartz cells, was used for the absorbance measurements during the development of this procedure.

An Ultrathermostat Model NBS water-bath (Gebrüder Haake K.G.) was used for the heat treatment.

Reagents and solutions

The following reagents and solutions were used: cefaclor (**I**), made by Eli Lilly, Lot No. 264BC6A (Switzerland); cefazolin sodium (**II**), made by Fujisawa, Lot No. 0070 (Japan); cefotaxime sodium (**III**), made by Roussel Uclaf, Cont. No. 6G0842 (France); cefoxitin sodium (**IV**), made by MS&D, Lot No. MEG-191 (USA); cefamandole nafate (**V**), made by Eli Lilly, Lot No. 5388J6, (UK); ammonium heptamolybdate, Merck, pro analysi, No. 1182 as a 0.5 M solution in water; sulphuric acid, Fluka, puriss., pro analysi, No. 84720 as a 0.5 M solution in water.

Standard solutions of **II**, **III**, **IV** and **V** were freshly prepared by dissolving the appropriate amount of each substance in water to form 2×10^{-3} M solutions (expressed

as the anhydrous substance). For **I**, the concentration of the standard solution was 5×10^{-4} M because of its greater molar absorptivity. The concentrations of the five standard solutions were verified by the official methods [9, 10].

Deionized and freshly prepared double-distilled water was used throughout the study.

Recommended procedures

For antibiotic substances. A suitable volume from each of the standard solutions of cephalosporins, to produce a final concentration between 10–100 μg ml^{-1} of **I**, 40–400 μg ml^{-1} of **II**, **III** and **V**, and 25–250 μg ml^{-1} of **IV** was pipetted quantitatively into a 50-ml test-tube.

Ten millilitres of $(NH_4)_6Mo_7O_{24}$ (2×10^{-3} M) and 5.0 ml of H_2SO_4 (0.5 M) were transferred into the same test-tube and mixed. If necessary, a sufficient quantity of water was added to produce a final volume near 23.0–24.0 ml and mixed again.

The prepared solution was placed for 15 min in the thermostatted water-bath (97°C ± 1°C). After completion of the heat treatment the contents of the test-tube were cooled quickly to room temperature, transferred carefully into a 25-ml volumetric flask and diluted to the mark with water, prior to mixing.

The absorbance of the blue solution was measured at 810 nm against a corresponding blank reagent which was treated similarly.

The quantity of each antibiotic was calculated from the corresponding calibration graph (absorbance at 810 nm versus concentration of cephalosporin) for each of the analysed antibiotics.

For injections. The assay was applied as described in the previous section, since the content of the injection vial, at least for the first four drugs (**I–IV**), was pure cephalosporin.

In the case of injections of **V** ("Mandokef", Eli Lilly), which contains 66 μg of sodium carbonate per g of injectable powder, no interference with the determination was observed, because this quantity of sodium carbonate was too small to change essentially the desirable acidity of the measured solution.

Results and Discussion

It has long been known that a blue solution is obtained by reduction of an acidified solution of Mo(VI) or by oxidation of an acidified solution of Mo(V).

The substances responsible for this blue colour are compounds in which the mean oxidation state of Mo is between 5+ and 6+; examples are $MoO_{2.0}(OH)$ and $MoO_{2.5}(OH)_{0.5}$.

These "blue compounds" that contain both oxide and hydroxide appear to represent an entire series of "genotypic" compounds (i.e. having the same basic structure but differing in the charges on cations and anions), with $MoO(OH)_2$ (olive-green colour) as one limit and MoO_3 as the other [6, 7].

Thus the colour of a reduced solution of Mo(VI) changes between emerald-blue and deep ultramarine-blue, as a function of the variation in the concentration of each absorbent species, which arises from the reduction of the Mo(VI). This variation in colour results in the simultaneous change of the λ_{max} of the treated solution. Thus, in previous similar papers, the λ_{max} of the measured solution was 684–697 nm for a H_2SO_4 concentration of 0.05 M, a $Mo_7O_{24}^{6-}$ concentration of 0.15% m/v (in the final solution)

and a heating treatment in boiling water for 30 min [2]. The λ_{max} changed to 670 nm for a H_2SO_4 concentration of 9 M, a $Mo_7O_{24}^{6-}$ concentration of 10% m/v (also in the final solution) and a heating treatment at 91.5°C for 15 min [3].

In the present work all the absorbance measurements were carried out at 810 nm.

The author believes that this difference between the values of the λ_{max}, owing to the above mentioned qualitative and quantitative variations of the absorbent species, is the consequence of the different conditions of the reduction of the Mo(VI). Kriss *et al.* [8] have shown that the λ_{max} of a reduced acidified solution of Mo(VI) is also a function of the nature of the reducing agent, mineral or organic.

Absorption spectra

The absorbance of the solution of each cephalosporin treated by the above procedure, was scanned in the region 550–850 nm against a corresponding blank reagent which was treated similarly. The scan speed was 50 nm min^{-1}. The resulting absorbance curves are illustrated in Fig. 1.

The apparent molar absorptivity (ϵ) values for each of these antibiotics were: 4.26×10^3 for **I**; 1.15×10^3 for **II**; 1.10×10^3 for **III**; 2.00×10^3 for **IV** and 1.15×10^3 for **V**, at 810 nm. These values of ϵ are the means of five determinations from four solutions of different concentrations of each cephalosporin.

The stability of the colour of the solution which resulted under the specified conditions, was remarkable. The tone and the intensity of the colour remained unchanged for more than 4–6 h.

Calibration graphs

The calibration graphs for the determination of analysed cephalosporins were obtained under the proposed experimental conditions.

A linear relationship was observed between absorbance A at 810 nm and concentration C, over the range 10–100 μg ml^{-1} for **I**, 40–400 μg ml^{-1} for **II**, **III**, **V**, and

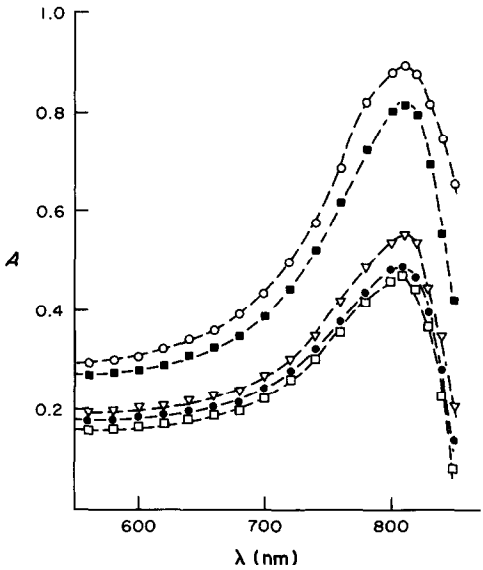


Figure 1
Absorption spectra of cephalosporins-paramolybdate anion reaction product. **I**, 2.1×10^{-4} M (○); **II**, 4.2×10^{-4} (●); **III**, 4.2×10^{-4} M (□); **IV**, 4.2×10^{-4} (■); **V**, 4.2×10^{-4} (▽).

Table 2
Regression equations with correlation coefficients, RSD values and the Sandell's sensitivity

Cephalosporin	Regression line equation	SD in gradient	Correlation coefficient (r)	RSD % (n = 10)	Sandell's sensitivity ($\mu g\ cm^{-2}$)
I	$A = 11.59 \times 10^{-3}C - 1.3 \times 10^{-3}$ (n = 8)	5.9×10^{-5}	0.9999	1.6 (at 50 $\mu g\ ml^{-1}$)	0.09
II	$A = 2.39 \times 10^{-3}C + 7.8 \times 10^{-3}$ (n = 8)	2.1×10^{-5}	0.9998	1.3 (at 200 $\mu g\ ml^{-1}$)	0.41
III	$A = 2.42 \times 10^{-3}C + 4.3 \times 10^{-3}$ (n = 8)	9.1×10^{-5}	0.9999	0.7 (at 200 $\mu g\ ml^{-1}$)	0.43
IV	$A = 4.59 \times 10^{-3}C - 5.2 \times 10^{-3}$ (n = 10)	4.6×10^{-7}	0.9999	1.1 (at 125 $\mu g\ ml^{-1}$)	0.22
V	$A = 2.5 \times 10^{-3}C + 1.0 \times 10^{-3}$ (n = 8)	7.6×10^{-5}	0.9999	1.2 (at 200 $\mu g\ ml^{-1}$)	0.44

25–250 μg ml^{-1} for **IV** in the final solutions. For these concentrations Beer's law was obeyed.

The regression line equation for absorbance A against concentration C (in μg ml^{-1}), the standard deviation in gradient (SD), the correlation coefficient (r), the relative standard deviation % (RSD % for $n = 10$) and the Sandell's sensitivity are shown in Table 2.

Application to the quality control of cephalosporins

The new procedure has been applied to the chemical assay of the cephalosporins in pure forms and in commercial pharmaceutical injections. Table 3 gives the results obtained by simultaneous application under industrial conditions of the proposed procedure and an official method.

Table 3
Determination of cephalosporins in pure form and in pharmaceutical preparations by the proposed procedure compared with the official methods

Samples	Proposed method Recovery*	RSD (%)	Official method Recovery*	RSD (%)	t†	Source of official method‡
I In pure form	100.5	1.26	99.5	2.36	0.92	USP XXI
Ceclor (Eli Lilly) Inj.	101.5	0.60	102.6	1.47	0.64	pp. 169–170
II In pure form	98.0	1.56	100.0	1.41	1.58	USP XXI
Vifazolin (Fujisawa) Inj.	104.0	1.36	104.9	1.03	1.23	pp. 174–175
III In pure form	100.8	2.08	101.1	1.77	0.27	USP XXI
Claforan (Roussel) Inj.	103.2	1.16	103.4	1.06	0.30	p. 176
IV In pure form	96.7	1.54	98.3	1.44	0.82	USP XXI
Mefoxil (MS&D)Inj.	104.7	0.75	106.0	0.84	1.55	pp. 176–177
V In pure form	100.0	1.41	98.6	2.31	1.28	U.S.CFR§ Title 21
Mandokef (Eli Lilly) Inj.	104.8	1.02	103.4	1.09	2.17	Part 442.8

*Means of six determinations.
†t = Calculated t-value (the theoretical value is $t = 2.23$, for 10 d.f. at $P = 0.05$).
‡USP XXI = U.S. Pharmacopeia XXI [9].
§U.S.CFR = U.S. Code of Federal Regulations [10] (iodometric titration procedure).

The same batch of each drug was analysed and the performance of the recommended procedure was assessed by calculation of the t-values.

Thus a mean value of $t = 1.07$ was obtained showing the absence of a systematic error in the method since the corresponding tabulated t-value for 10 d.f. and for the 95% confidence level is $t = 2.23$.

This comparison shows that there is no significant difference between the results of the recommended procedure and those of the official methods. This confirms that there is no evidence of interference with the procedure by degradation products.

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