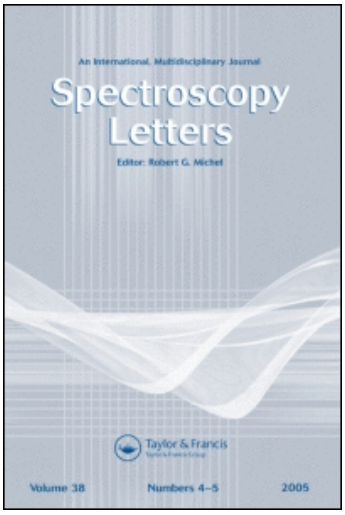

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Stability-Indicating Method for the Determination of Some Cephalosporines in the Prescence of Degradation Products

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Stability-Indicating Method for the Determination of Some Cephalosporines in the Prescence of Degradation Products

Keywords: Spectrophotometry; pharmaceutical analysis; stability indicating method; determination of Cephalosprines; drug formulation.

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ABSTRACT: A rapid and convenient spectrophotometric method is described for the quantitative determination of some of Cephalosporines Cefadroxil, I Cephradine II and Cefaclor III. The proposed method depends upon the conversion of these compounds to the corresponding piperazine-2,5 dione derivatives by heating in an alkaline sorbitol-zinc ion solution for 10-25 minutes at 90°C and subsequent treatment of these derivatives with 0.1 N sodium hydroxide to obtain highly absorbing products with λ_{max} at 345 nm for I & II and at 334 nm for III using zero order absorption curve. Using first derivative spectrum (D_1) for III the λ_{max} is at 362 nm. The method was found to be free of the interference from polymerization and other degradation products. Its application to assess the stability of the Cephalosporines was demonstrated. Verification of Beer's Law showed

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linearity at concentrations of 12.5-87.5 $\mu g\text{-}ml^{-1}$, 12.5-125 $\mu g\ ml^{-1}$ and 6.7-66.7 $\mu g\ ml^{-1}$ for I, II and III respectively with mean accuracies 100.37 ± 0.72%, 100.45 ± 0.87% and 99.63 ± 1.97% when using zero order absorption curve. Using D1 for III gave linearity at concentration range 42-114 $\mu g.ml^{-1}$ with mean accuracy of 99.54 ± 0.77%.

The proposed method is applied successfully for the determination of Cephalosprines in bulk powder and in pharmaceutical preparations. The results obtained by the proposed method are statistically analysed and compared with those obtained by applying the USP XXII method.

Several methods have been reported for the determination of I including colorimetric [1-3], HPLC [4], TLC [5] and differential pulse polarography [6].

Fluoremetric [7], derivative Spectrophotometric [8], UV absorptiometric [9], Colorimetric [10,11] and HPLC [12] methods have been reported for the determination of II.

Some analytical methods have been reported for the determination of III. These include HPLC [13-15], Colorimetric [16,17], TLC [5] and UV absorptiometric [18] methods.

The purpose of this manuscript is to determine the previously mentioned drugs in the presence of their degradation products by a selective method.

EXPERIMENTAL

Materials:

* Cefadroxil, kindly supplied by Kahira Co-Egypt. The purity of the sample was found to be 100.00 ± 0.22% according to the USP XXII method [19] I.
* Cephradine, kindly supplied by Bristol-Myers Squibb Egypt. The purity of the sample was found to be 99.80 ± 0.56% according to the USP XXII method II.
* Cefaclor, kindly supplied by Epico, Cairo-Egypt. The purity of the sample was found to be 100.50 ± 0.15% according to the USP XXII method III.

* Deuricif capsules (Kahira Co.), batch No. 1224. Each capsule claimed to contain 500 mg Cefadroxil.
* Ibidroxil syrup in powder form (APIC), batch No. 928. Each 5 ml syrup claimed to contain 125 mg Cefadroxil.
* Velosef capsules (Spuibb), batch No. J51914. Each capsule claimed to contain 500 mg Cephradine.
* Velosef vials (Squibb), batch No. H51684. Each vial claimed to contain 1 gm Cephradine.
* Ultracef vials (Misr), batch No. 203095, 204095. Each vial claimed to contain 250 mg and 1 gm Cephradine respecttively.
* Cefaclor syrup (Eipico), batch No. AD2130. Each 5 ml syrup claimed to contain 125 mg Cefaclor Reagents.

Reagents:

All chemicals used throughout this work were analytical grade:

* Sodium Hydroxide: 0.1 N aqueous solution.
* Carbonate buffer [20] 0.2 N, pH 9.6 $\pm$ 0.1.
* Sorbitol reagent, 25% m/v of sorbitol in a 0.2 M carbonate buffer containing 20 $\mu g.ml^{-1}$ of zinc sulphate, the pH of the final solution being 9.6 $\pm$ 0.1.

Apparatus:

* DU-7 Spectrophotometric Beckman.
* pH meter (Philips).

Standard Solution:

Transfer 50 mg accuratly weighed of each of I, II & III; each in 25 ml volumetric flask dissolve in few ml distilled water and complete to volume. Each 1 ml of each solution contains 2 mg.

Procedure:

A. Bulk powder

Transfer 0.5 ml twice (A) and (B) of standard solution of drug into two separate test tubes. Add to (A) 5.0 ml of the sorbitol reagent, mix (B) with 5.0 ml of water, immerse in a water bath at 90°C for 10, 15, and 20 min. for I, II and III respectively. Cool, pipette 0.5 ml of each (A) and (B) of each drug solution into cuvette containing 2 ml of 0.1 M sodium hydroxide. Use (B) as the reference and measure the absorbances for I, II at 345 nm, and for III at 334 nm. Measure D_1, at 362 nm. for III. Determine the concentration of the Cephalosporines from $A^{1\%}_{1cm}$ or from the regression equation or from a calibration curve analysed simultaneously, in concentration range of 12.5-87.5 $\mu g.ml^{-1}$ for I, 12.5-125 $\mu g.ml^{-1}$ for II and 6.7-66.7 $\mu g.ml^{-1}$ for III. For III D_1 calibration graph concentration range from 42-114 $\mu g.ml^{-1}$.

B. Dosage forms

1. For Capsules:

Empty the content of 5 capsules mix well and take a weight equivalent to one capsule. Dissolve in 50 ml distilled water in volumetric flask. Transfer an aliquote equivalent to 50 mg in 25 ml volumetric flask and dilute with water to obtain a concentration equivalent to 2 $mg.ml^{-1}$ and proceed as under bulk powder starting with the words transfer 0.5 ml twice (A) and (B)....

2. For Oral suspension:

Constitute the container for oral suspension as directed in labelling. Filter through a filter paper and dilute quantitatively with water to obtain a solution containing about 2 $mg.ml^{-1}$. Proceed as under bulk powder starting with the words transfer of 0.5 ml twice (A) and (B).....

3. For Injections:

Constitute the sterile vial in a volume of water, accurately measured, corresponding to the volume of solvent specified in the labelling. Withdraw all the contents and dilute quantitatively with water to obtain a solution containing about 2 $mg.ml^{-1}$ and proceed as under bulk powder starting with the words transfer 0.5 ml twice (A) and (B).....

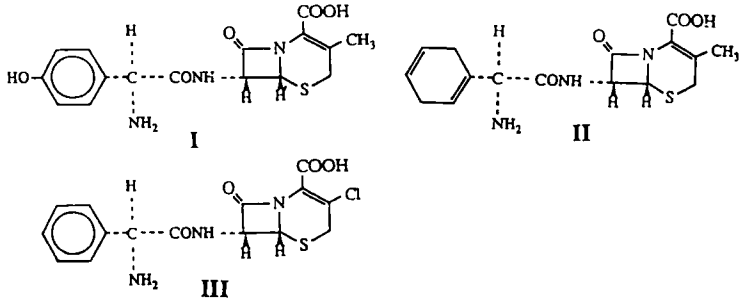
RESULTS AND DISCUSSION

The proposed method depends upon the determination of cephalosporines which contain an intact B-Lactam and a free amino group in the side chain of the molecule in prescence of polyhydric alcohol. These compounds are expected to undergo estrification followed by aminolysis and cyclization to form piperazine- 2,5 dione derivative. This proposed method was based on the reaction that suggested and proved by Bundgoard et al [21,22] for penicillins having B-lactam and free amino group in the side chain. Several penicillins of this type were found to form penicilloyl esters in prescence of various carbohydrates (glucose and fructose) and polyhydric alcohol (sorbilol and glycerol). This is rapidly followed by intramolecular aminolysis with the formation of piperazine 2,5 dione derivative [21,22].

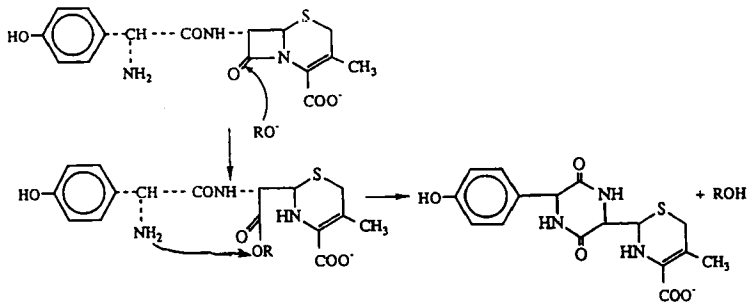
Accordingly Cephalosporines I, II and III containing the same functional groups namely B-Lactam and free Amino group in the side chain of the molecule were chosen.

The reaction is suggested to take place according to the following scheme, which is based on the postulations of Bundgoard et al [21,22] for pencillins.

In this work several polyhydric compounds e.g. sorbitol, fructose and glucose were tried. Sorbitol was found to give the highest absorbance and more reproducible results than the other examined carbohydrates and the experimental conditions were adjusted.



Structure 1



Structure 2

The optimum concentration of sorbitol was found to be 25 mg% in carbonate buffer. The rate of the reaction has been found to increase proportionaly with increase of pH up to pH 9.6 $\pm$ 0.1. Furthermore, it was observed that at pH value greater than pH 9.7 the formed piperazine dione showed a slow degradation (Fig. 1). Therefore, carbonate buffer of pH 9.6 $\pm$ 0.1 was chosen. When using phosphate buffer low absorbance values were obtained.

Different sodium hydroxide concentrations were tried, the best concentration was 0.1 N where low blank absorbance values were obtained. The maximal catalytic effect

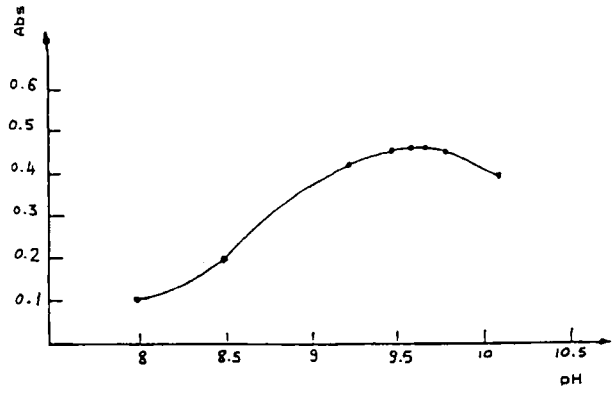


Figure 1: Effect of pH on reaction of Cephalosprines (40 μg ml^{-1}) with the Sorbitol reagent at 90°C

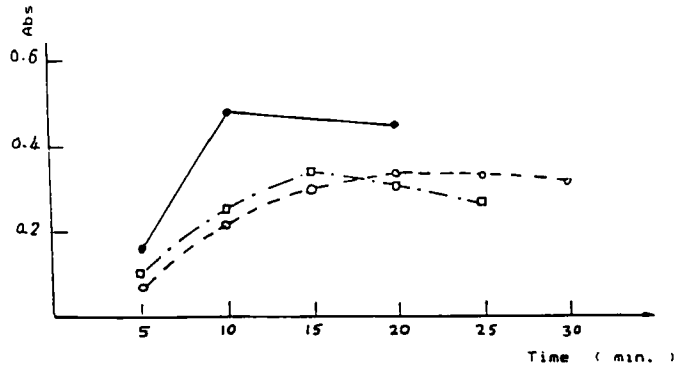


Figure 2: Effect of time of heat on the formation of piperazine dione derivative (40 μg. ml^{-1} of each) — Cefadroxil p-pCephradine, o-o Cefaclor .

was reached when the zn(II) concentration was 20 μg.ml^{-1}. The effect of temperature and time is shown in (Fig. 2,3).

On applying the proposed method to different concentrations of authentic samples of I, II and III a linear correlation was obtained between the absorbances and the

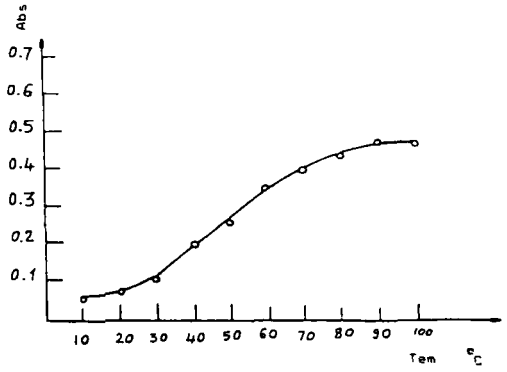


Figure 3: Effect of temperature on reaction of Cephalosporines (40 μg. ml^{-1}) with the Sorbitol reagent.

Table 1: Determination of authentic samples of Cefadroxil, Cephradine, and Cefaclor using the proposed method and the official USP 22 method.

	Cephadroxil		Cephradine		Cefaclor		
	The proposed method	USP 23	The proposed method	USP 23	The proposed method		USP 23
					Zero-order	First derivative	
Concentration range μg.ml^{-1}	12.5 - 87.5		12.5 - 125		6.7 - 66.7	42 - 114	
Mean recovery* % ± S.D.	100.37 ± 0.73	99.64 ± 0.52	100.45 ± 0.87	99.92 ± 1.3	99.63 ± 1.09	99.54 ± 0.77	99.25 ± 1.43
F.calculated	1.93		2.47		1.7	3.46	
t.Calculated	1.97		0.67		0.51	0.43	

* The avarage of six experiments
F.tabulated (5.1)
t.tabulated (2.228)
P = 0.05.

Table 2: Determination of Cefadroxil, Cephradine and Cefaclor in pure form and in pharmaceutical preparations by the proposed method* and the offical method USP 23.**

Preparations	The proposed method found % ± C.V	Standard addition Recovery % ± C.V.	Official method USP 23 found % ± C.V.
I. Cefadroxil Pure Sample			99.64 ± 0.52
Durecif Capsule 500 mg/CAP	99.80 ± 0.53	99.98 ± 0.23	99.7 ± 1.16
Ibidroxil Capsule 250 mg/CAP	99.95 ± 0.14	100.93 ± 0.35	98.92 ± 0.75
Ibidroxil Syrup 125 mg/5 ml	100.72 ± 0.11	100.02 ± 0.52	99.05 ± .53
II. Cephradine Pure Sample			99.92 ± 1.37
Velosef Capsule 500 mg/CAP	99.83 ± 0.23	99.93 ± 1.3	100.11 ± 0.96
Velosef Vial 1 gm/Vial	99.60± 0.71	100.46 ± 0.72	100.32 ± 1.54
Ultracef Vial 250 mg/Vial	100.32 ± 0.05	100.28 ± 0.74	99.81 ± 0.88
Ultracel Vial 1 gm/Vial	100.2 ± 0.11	99.25 ± 0.38	99.13 ± 0.51
III. Cefaclor Pure Sample			99.25 ± 1.43
Cefaclor Syrup 125 mg/ 5 ml	99.64 ± 0.53	100.67 ± 0.47	98.74 ± 1.12

* mean of five determinations

** mean of three determinations

concentrations from which the linear regression equation was calculated.

$Y = 0.0001 + 0.01\ 3C$ $r = 1.0$ For I

$Y = 0.009C-0.02$ $r = 0.9999$ For II

$Y = 0.028 + 0.01\ C$ $r = 0.9996$ For III

in zero absorbance curve and

$D_1 = 0.0002C-0.0035$ $r = 1.006$ For III in D_1 curve

Table 3: Determination of cefacdroxil, Cephradine and Cefacler in presence of the degradation products.

Amount added** mg	Recovery* %					
	Cefadroxil		Cephradine		Cefaclor	
	0.1N H_2SO_4	0.001 N NaOH	0.1 N H_2SO_4	0.001 N NaOH	0.1 N H_2SO_4	0.001 N NaOH
0.5	99.21	99.35	100.37	99.26	99.15	99.67
1	100.55	99.62	99.22	99.57	00.09	99.29
2	99.87	100.36	100.05	100.70	99.59	99.44

* Average of three experiments.

** Amount added per 2 mg of drug.

where Y is absorbance, C is concentration and r is correlation coefficient and D_1 is the first derivative absorbance. $A^{1\%}_{1cm}$ was determined for the three analyz ed drugs and found to be 127.6 for I, 86.5 for II and 109.4 for III respectievly. The $D^{1\%}_{1cm} \times 10^2$ for III was calculated also and found to be 153.

The proposed method was applied for the analysis of authentic drug samples (Table 1) and pharmaceutical preparations (Table 2). The data shows that there is no significant difference between the proposed and the official method. The validity of the proposed method was assessed by applying the standard addition technique. The results are presented in (Table 2).

Since the formation of the piperazine dione derivative is dependent on both an intact B-Lactam ring and an intact amino side chain group in the cephalosporines molecule, no interference in the method by degradation products should be expected. This was confirmed when degradation of celphalosporine samples was carried out by dissolving the samples (0.1% of each) in 0.1 N sulphuric acid and 0.001 N sodium hydroxide and stored at 90°C for 3 hours. No Cephalosporines were detected by the method in the degraded samples. Freshly prepared Cephalosporines aqueous solution (0.2% of each) were mixed with different ratios with the prepared degraded sample solutions. The mixtures were analysed by the proposed method. The percentage recoveries of added Cephalosporines were within the range 99.1 - 100.7%, (Table 3).

From the statistical analysis comparison of the results obtained by applying the proposed method and the USP XXII method it was found that there is no significant difference between the two methods (Table 1).

The advantage of the suggested method over other methods is that, with the exception of the HPLC method, it determines only the intact drug in the presence of the degradation products. Moreover, the method is inexpensive, rapid and sensitive.

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