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Accelerated stability studies on Rocephin by high-efficiency liquid chromatography

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Summary

The stability of Rocephin in aqueous solutions was studied for different pH values in research into accelerated instability. The pH range studied was 2.5, 4.5, 5.5, 6.5, 7.4 and 8.0 adjusted to an ionic strength of $\mu = 0.5$; each of these values was subjected to temperatures of 50, 60, 70 and 80°C. From the experimental values found for the concentrations of undegraded Rocephin as a function of time it was seen that the degradation process of this cephalosporin follows first-order kinetics.

The technique used for the determination of Rocephin was high-efficiency liquid chromatography, using a reverse phase (RP-18) as the stationary phase.

Linear relationships were established for each pH studied, between the logarithms of the experimental degradation constants and the reciprocals of the absolute temperatures, obtaining correlation coefficients greater than 0.91.

These values were used to calculate the values of the degradation constants, half-lives and expiry date for each pH at temperatures of 37, 20 and −4°C. Simultaneously, the activation energy and the logarithm of the frequency factor were determined, giving average values of 13.11 kcal/mol and 5.62, respectively.

The values obtained for the expiry date at 20°C, greater than 7 h for all the pH values mean that in terms of pH-regulated degradation this antibiotic may be used in the normal perfusion liquids.

Introduction

Rocephin is a semi-synthetic β-lactam antibiotic recently introduced for parenteral use and which has a wide antimicrobial spectrum. It is also known as Ro-13-9904,

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ceftriaxone or cefatriaxon and chemically is the disodium salt of {(6R,7R)-7-[2-(2-amino-4-thiazolyl)-2-(Z-methoxyimino)-acetamido]-3-[(2,5-dihydro-6-hidroxy-2-methyl-5-oxo-as-triazine-3-yl)thiomethyl]ceph-3-em-4-carboxylic acid} (Neu et al., 1981).

Several studies carried out in vitro have shown how its antimicrobial spectrum and potency are almost identical to cefotaxime (Bint et al., 1981; Eickhoff and Ehret, 1981). In vivo studies have also been carried out in mice experimentally infected with *Pseudomonas aeruginosa*; in this instance it was seen that Rocephin is more efficient that cefotaxime and cefoperazone in 8 of the 10 strains studied (Beskid et al., 1981).

The pharmacokinetics of Rocephin was studied after administration of a single i.v. dose of 500 mg in 6 healthy volunteers. The serum levels of Rocephin administered by this route follow an open two-compartment kinetic model with a half-life of the slow elimination phase of 8 h, considerably greater than that normally shown by other cephalosporins (Seddon et al., 1980).

The clinical efficiency of Rocephin has been studied in the treatment of various infections such as septicaemia (Nagler and Herten, 1981), pielonephritis (Giamarellou et al., 1981), meningitis (Narciso et al., 1981), pneumonia (Pichler et al., 1981) and gonorrhoea (Harder et al., 1981) among others, and satisfactory efficiency has been the rule.

The studies of accelerated instability, replacing the older techniques for studying degradation, have currently been consolidated in a method which guarantees the activity and integrity of the drugs in question. Such studies are most suitable when the drugs are in solution, where the physicochemical system is limited in duration.

The processes of instability may be attenuated or even anulled by turning to different techniques such as the introduction of antioxidants, antihydrolizers, etc., or liophilizing the preparation. However, it should be remembered that, just as in the case of the drug under consideration here, hospital practice frequently involves i.v. perfusion, dissolving the drug in media which do not always guarantee its stability throughout administration. This was the practical reason, apart from the mere desire to shed light on the stability of a recently introduced drug, which prompted us to carry out the present work.

Analytical technique

The analytical technique used in the determination of native Rocephin was high-efficiency liquid chromatography, using a Varian mod. 5000 chromatograph coupled to a Varian mod. 9176 register with a U.V.-visible variable wavelength spectrophotometer equipped with a fixed volume coil injector. The first step in the procedure was to check the wavelength at which the maximum absorbance for the whole pH range studied is produced. It was seen that for all the pH values the absorbance maximum was between 250 and 260 nm and the wavelength of the detector was therefore set at 254 nm.

Following this, the working conditions of the chromatograph were established as below.

Column: stationary phase R.P.-18, length 25 cm; internal diameter 0.46 cm.
Temperature: room temperature.
Mobile phase: 0.01 M sodium acetate–methanol (70/30 v/v).
Flow rate: 1 ml/min.
Chart rate: 1 cm/min.

Under these conditions, the retention time of Rocephin was seen to be 2.7 min.

Materials and Methods

The accelerated instability of Rocephin was studied in aqueous solutions buffered at different pH values (2.5, 4.5, 5.5, 6.5, 7.4 and 8.0) using Teorell and Stenhagen's citrate–phosphate–borate buffer and adjusting ionic strength to 0.5 in each case.

An initial solution of Rocephin at known concentration was prepared at the desired pH of the experiment to be carried out. From this solution aliquots of 5 ml were placed in glass vials under a stream of nitrogen which were then sealed. The aliquots were divided into 4 lots, and each of these was subjected to different temperatures in a thermostatted water bath. At previously programmed times the samples were removed from the bath and immediately plunged into an ice-water mixture. Following this, the vials were stored at $-20\,^{\circ}C$ until determination.

Theoretical

Experiments in studies on accelerated instability usually consist in determining a series of concentrations of the undegraded drug as a function of time, at a given pH and temperature. With the resulting data a determination is first made of the order of the degradation reaction, after which it is possible to determine the instability constant in such a way that as a function of the pH value, we will obtain a value of the instability constant for each temperature (Lachman et al., 1976).

By means of the Arrhenius equation, it is possible to relate constants and temperatures:

$$K = A \cdot e^{-E_a/RT}$$

where K = rate constants of the process, A = frequency factor, E_a = activation energy, R = gas constant (1.987 cal/deg · mol) and T = temperature in absolute degrees.

That is:

$$\log K = \log A - \frac{E_a}{2.303\,R} \cdot \frac{1}{T}$$

With the relationships established between the constants and the temperatures, it is possible to extrapolate the values of the inactivation constants at any other temperature, such as body temperature (37 °C), normal storage temperature (20 °C)

and freezer storage temperature (−4°C) for the same pH value. From these constants it is possible to determine the inactivation half-life; i.e. the time it takes to inactivate 50% of the initial drug. It is also possible to determine the expiry date if the drug is formulated in solution at that pH; i.e. the time 10% of the initial drug would take to degrade. Similarly, all determinations were repeated for the complete range of pH values studied.

It is classically recognized that the possible degradation mechanisms for cefalosporin antibiotics are: hydrolytic breakdown of the β-lactam ring by direct attack by water, and degradation caused by intramolecular participation of the nearby amide group on the C = 0 bond of the β-lactam ring.

Where a side-chain amide participates in the degradation of cefalosporins by an intramolecular reaction, the introduction of an electron-donating substituent into the side-chain would facilitate the breaking down of the ring, while an electron-withdrawing substituent would retard it. This theory necessitates the existence of an intermediate degradation product, highly reactive, which could be formed under physiological conditions of temperature and pH and which might play an important part in allergies to cephalosporins (Rattie et al., 1979).

Results and Discussion

Fig. 1 shows the initial results of undegraded Rocephin concentrations for each pH value and temperature as a function of time. Using a Hewlett-Packard 97 microcomputer it was possible to determine from these values that the degradation of Rocephin follows a first-order kinetics (Rey-Bellet and Etter, 1980).

Table 1 shows the values of the degradation rate constants obtained for the different pH values and temperatures in our study, together with the corresponding degradation half-lives and expiry date.

Fig. 2 shows the plot of the values obtained for the degradation constant at 60°C as a function of the pH value studied. Three clearly differentiated zones may be seen; a first zone at acid pH between 2.5 and 4.5, in which the constant scarcely varies with pH and which corresponds to the zone of maximum stability. There is a second zone in the pH range of 4.5–7.4 where the instability of Rocephin increases and this is followed by a third zone starting at pH 7.4 towards base values in which linearity disappears, possibly because at such high pH values there are different ionic species with different degradation rates, as has been described for other cephalosporins (Mariño and Dominguez-Gil, 1982).

With the results obtained for the inactivation constants at the experimental temperatures the Arrhenius equation was fitted, and with a least-squares program run on a Hewlett-Packard 97 the regression slopes plotted in Table 2 were obtained, together with the corresponding correlation coefficients. Table 2 also shows the values of the activation energy and the logarithm of the frequency factor for each of the pH values studied.

By extrapolation of the linear regression slopes for each pH value it was possible to obtain the theoretical values of the degradation constants, the half-lives and the

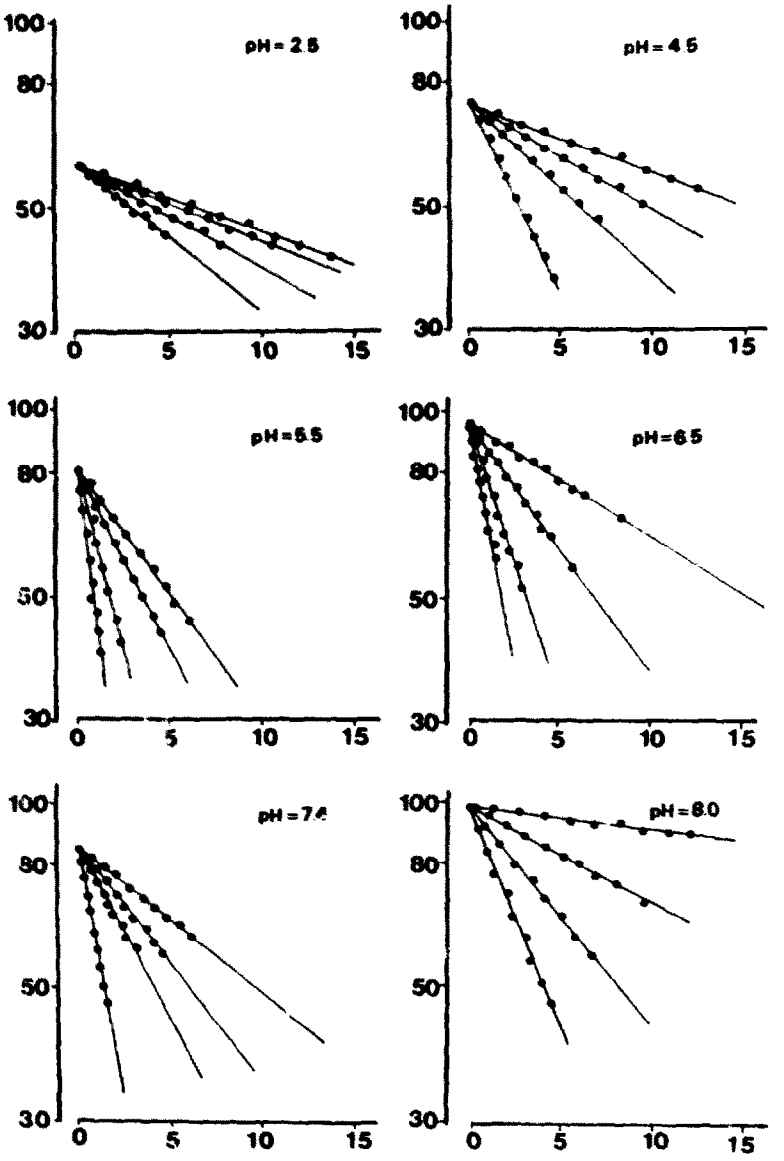

Fig. 1. Non-degraded Rocephin concentrations (μg/ml) as a function of the pH and temperatures values studied versus the time.

expiry date of the drug for temperatures of 37, 20 and −4°C, as shown in Table 3.

A detailed study of the regression equations of Table 2 shows that the different slopes corresponding to different pH values intersect at various points. In other words, each of these intersections corresponds to a temperature at which the degradation rate constant is the same for several pH values. This temperature is 'critical' since the rate of the process is reversed on either side of this value. Consequently, the influence of pH on the degradation rate is shown differently at room temperature than at higher temperatures and this has bearing on storage and stability criteria. This is undoubtedly because the reaction mechanisms are a

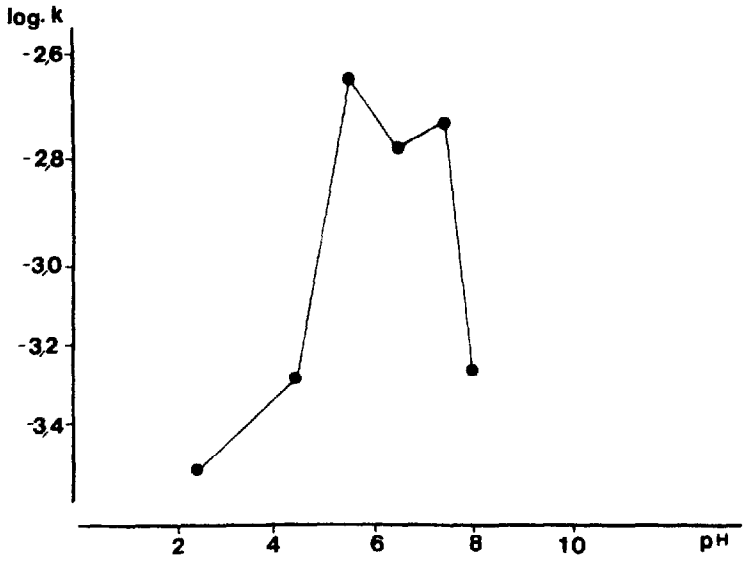

Fig. 2. Plot of the degradation of Rocephin at 60°C as a function of pH values.

TABLE 1

ROCEPHIN: DEGRADATION CONSTANTS, HALF-LIFE AND $t_{90\%}$ OBTAINED AS A FUNCTION OF THE EXPERIMENTAL pH AND TEMPERATURE VALUES

pH	T (°C)	K (min^{-1})	$t_{1/2}$ (h)	$t_{90\%}$ (h)
2.5	50	0.0004	28.87	4.37
	60	0.0004	28.87	4.37
	70	0.0007	16.50	2.50
	80	0.0009	12.83	1.94
4.5	50	0.0004	28.87	4.37
	60	0.0006	19.25	2.92
	70	0.0011	10.64	1.59
	80	0.0026	4.44	0.67
5.5	50	0.0015	7.70	1.17
	60	0.0021	5.50	0.83
	70	0.0039	2.96	0.45
	80	0.0064	1.80	0.27
6.5	50	0.0010	11.55	1.75
	60	0.0016	7.22	1.09
	70	0.0040	2.89	0.44
	80	0.0075	1.54	0.23
7.4	50	0.0010	11.55	1.75
	60	0.0018	6.42	0.97
	70	0.0018	6.42	0.97
	80	0.0059	1.96	0.30
8.0	50	0.0002	57.75	8.75
	60	0.0006	19.25	2.92
	70	0.0013	8.88	1.35
	80	0.0024	4.81	0.73

TABLE 2

ROCEPHIN: RESULTS OBTAINED AFTER ARRHENIUS TREATMENT

pH	Regression equation	Correlation coefficients	Statistical significance levels	Activation energy	log of frequency factor
2.5	$\log K = 1.1153 - 1.4736 \cdot \frac{1}{T} \cdot 10^3$	0.9401	< 0.001	6.74	1.1153
4.5	$\log K = 6.0396 - 3.0659 \cdot \frac{1}{T} \cdot 10^3$	0.9823	< 0.001	14.03	6.0396
5.5	$\log K = 4.7478 - 2.4559 \cdot \frac{1}{T} \cdot 10^3$	0.9919	< 0.001	11.24	4.7478
6.5	$\log K = 7.6106 - 3.4406 \cdot \frac{1}{T} \cdot 10^3$	0.9915	< 0.001	15.74	7.6106
7.4	$\log K = 5.0755 - 2.6184 \cdot \frac{1}{T} \cdot 10^3$	0.9165	< 0.001	11.98	5.0755
8.0	$\log K = 9.1305 - 4.1305 \cdot \frac{1}{T} \cdot 10^3$	0.9950	< 0.001	18.90	9.1305

K = degradation constants; T = temperature (in degrees Kelvin).

TABLE 3

ROCEPHIN: RESULTS OBTAINED AFTER EXTRAPOLATION TREATMENT FOR THE DEGRADATION CONSTANT, HALF-LIFE AND $t_{90\%}$ AT 37, 20 AND −4°C

pH	T (°C)	K (min^{-1})	$t_{1/2}$ (h)	$t_{90\%}$ (h)
2.5	−4	4.3380×10^{-5}	266.25	40.34
	20	1.2192×10^{-4}	94.73	14.41
	37	2.3003×10^{-4}	50.21	7.64
4.5	−4	4.3876×10^{-6}	2632.42	398.88
	20	2.7679×10^{-5}	306.54	46.62
	37	1.4115×10^{-4}	81.33	12.45
5.5	−4	4.1485×10^{-5}	278.41	42.18
	20	2.3227×10^{-4}	49.73	7.56
	37	6.6911×10^{-4}	17.26	2.63
6.5	−4	6.6096×10^{-6}	1747.45	264.75
	20	7.3824×10^{-5}	156.45	23.79
	37	3.2501×10^{-4}	35.54	5.40
7.4	−4	2.1957×10^{-5}	526.03	79.70
	20	1.0000×10^{-4}	115.50	17.57
	37	4.0000×10^{-4}	28.87	4.39
8.0	−4	6.3861×10^{-7}	18086.01	2740.55
	20	1.0789×10^{-5}	1070.53	162.82
	37	6.3944×10^{-5}	180.63	27.47

function of pH and are also temperature-dependent.

The determination of the expiry date at different pH values and temperatures allowed us to check that for a temperature greater than 20°C the expiry date was greater than 7 h for all the pH values studied. This means that Rocephin in as far as degradation is concerned may be used in solution in conjunction with the customary perfusion fluids.

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