Journal of Pharmaceutical & Biomedical Analysis
Vol. 6, No. 1, pp. 23–28, 1988
Printed in Great Britain

0731–7085/88 $3.00 + 0.00

Spectrophotometric determination of the stability of an ampicillin–dicloxacillin suspension

V. GIRONA, C. PACAREU, A. RIERA, R. POUPLANA, M. CASTILLO* and J. BOLÒS

Departmento de Físico-Química Aplicada, Facultad de Farmacia, Universidad de Barcelona, Núcleo Universitario de Pedralbes, 08028 Barcelona, Spain

Abstract: The degradation rate constants for ampicillin and for dicloxacillin in the suspension filtrate, and their solubility coefficients (at 25°C) were determined by spectrophotometry employing a multicomponent computer program. The shelf life of the ampicillin–dicloxacillin suspension was then determined in terms of the stability of ampicillin, the least stable component.

Keywords: *Ampicillin, dicloxacillin, shelf life, stability study, spectrophotometry.*

Introduction

Ampicillin and dicloxacillin are frequently formulated together, usually in the concentration ratio of 2 : 1, because a synergistic antibacterial effect is produced which is related to their different affinities towards plasma proteins, and the resistance of dicloxacillin to penicillinases [1].

This kinetic study was based on the calculation of the specific degradation rate constant for each penicillin in the mixture using a Hewlett–Packard 8451A spectrophotometer fitted with a multicomponent analysis module. The calculated values permitted the determination of the shelf life for the suspension, which, since it contains more than one active principle, is based upon the penicillin experiencing the faster degradation process.

Materials and Experimental Methods

Substances: ampicillin trihydrate of pharmaceutical quality (Impex Quimica S.A.); sodium dicloxacillin monohydrate of pharmaceutical quality (Antibióticos S.A.).

Apparatus: spectrophotometer Hewlett–Packard 8451A with a multicomponent analysis module; microcomputer Tektronik 4051.

Analytical method

In order to simultaneously obtain the concentrations of both antibiotics present in the suspension, a multicomponent computer program incorporated into the spectro-

*Deceased.

photometer has been used. The total antibiotic concentration, necessary to carry out the multicomponent analysis, was obtained by the imidazole method [2] following acetylation of both antibiotics. This step was necessary to overcome the difficulty produced by the $-NH_2$ group in the ampicillin if the standard imidazole method had been used [3]. In the case of the dicloxacillin the acetylation process was unnecessary [4]. However, several experiments showed that this process did not affect its UV-spectrum nor the absorbance maximum position (345 nm). Moreover, it has been found that the absorbances of the mercuric mercaptides obtained from pure ampicillin and pure dicloxacillin were always proportional to the quantities of antibiotic employed.

The multicomponent analysis program permitted the concentration of each of the components present in the mixture to be obtained without prior separation. In order to carry out such an analysis it was necessary to obtain the spectra of the pure components at several concentrations. This data was then retained in the computer memory. The concentrations of the pure components were chosen so that the mean absorbances of the components, in the wavelength interval used, were of the same order. The computer program carried out an optimal fit between the spectra of the sample to be analyzed and those of the pure components already stored in the computer. As a result the composition of the mixture was obtained. The goodness of fit was measured by means of the standard deviation which was compared with the possible deviations expected as a function of the noise corresponding to the interval employed [5].

The optimum interval corresponded to that in which the main differences between the two spectra occurred. In the present case the optimum wavelength interval was found to be 320–360 nm (Fig. 1). In this interval it was also found that the proportionality between concentrations and absorbances held in all cases.

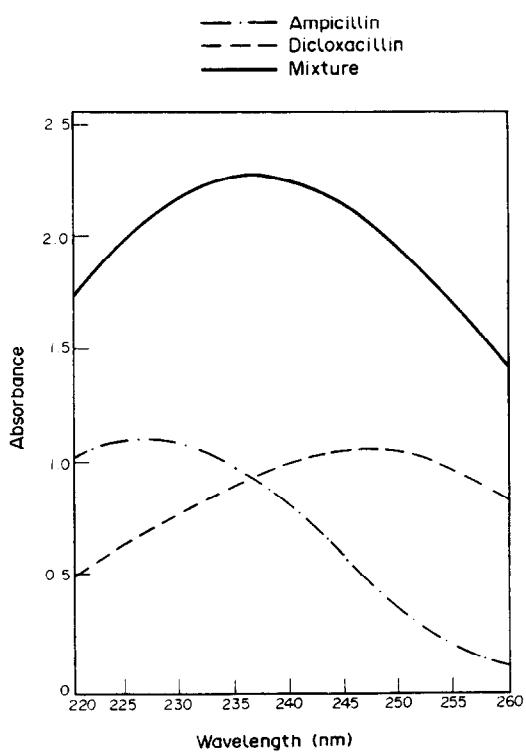

Figure 1
A typical UV-spectrum (320–360 nm) of the penicillin mixture used to perform the multicomponent analysis.

Results and Discussion

Kinetic study of the ampicillin–dicloxacillin suspension

In order to apply the Arrhenius equation in the study of the accelerated stability study of the suspension it is necessary to know the degradation reaction order corresponding to each of the penicillins present in the mixture.

The ampicillin suspension used to carry out the present determinations was prepared using 2.5% w/v ampicillin, although its solubility is about 0.7% w/v in water. The quantity of dicloxacillin in the suspension was less than its solubility in water [6].

The experiment was carried out at 35°C. The suspension was prepared by mixing in a 10 ml flask the following substances: ampicillin (0.2500 g), dicloxacillin (0.1250 g), saccharose (5.6262 g) and aerosil 200 (0.0187 g). A 0.1 M acetic acid–sodium acetate buffer solution (pH = 5.6, ionic strength, μ = 0.5) was added to give a final volume of 10 ml. The analysis was performed on samples removed at different intervals which were then diluted to 100 ml and filtered. By means of the multicomponent computer program the concentrations of each penicillin in the mixture were obtained, and the corresponding graphs produced (Figs 2, 3).

These figures show that both penicillins in the mixture were degraded at a constant rate, indicating that the reactions are zero order. It must be pointed out that the

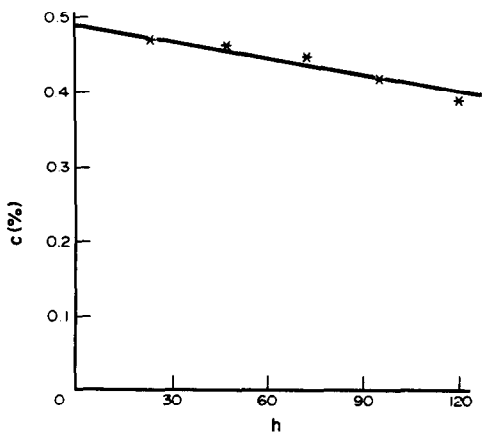

Figure 2
Stability of ampicillin in the suspension at 35°C.

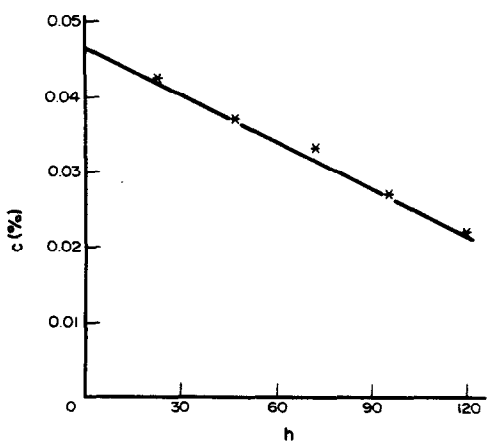

Figure 3
Stability of dicloxacillin in the suspension at 35°C.

dicloxacillin degradation process may also be represented as a first order reaction, but in this case the standard deviation is higher than that obtained assuming a zero order reaction. Consequently, the Arrhenius equation cannot be applied to obtain the zero order rate constant at 25°C, which is necessary to compute the shelf life. To apply this equation one must find the rate constants at several temperatures for the suspension filtrate, which shows a first order degradation process.

Kinetics of the ampicillin–dicloxacillin filtrate

The suspension was prepared as described above and filtered following agitation. The analysis was carried out by means of the imidazole method on an aliquot of the filtrate and the remainder divided into samples being maintained at 35, 40, 50 and 60°C. Samples were taken at different times for each temperature and analyzed in order to follow the kinetic process. From the experimental absorbances the concentrations of both penicillins were calculated as indicated previously. The rate constants for the ampicillin and dicloxacillin were then calculated at each one of the temperatures used by means of a least squares method including weighted regression [7] (Tables 1, 2).

Using the experimental values obtained at 35, 40, 50 and 60°C, the Arrhenius equation permits the calculation of the first order rate constant (k_1) at 25°C for each one of the penicillins present in the mixture (Table 3).

Table 1
Ampicillin present in the suspension filtrate

T = 35°C		T = 40°C		T = 50°C		T = 60°C	
ln A	t	ln A	t	ln A	t	ln A	t
−0.546	0.00	−0.546	0.00	−0.669	0.00	−0.571	0.00
−0.582	6.16	−0.607	6.16	−0.691	1.00	−0.557	0.75
−0.539	21.25	−0.721	21.25	−0.744	2.00	−0.669	1.25
−0.779	44.33	−0.941	44.33	−0.787	3.06	−0.685	1.75
−0.872	51.83	−1.008	51.83	−1.158	18.33	−0.734	2.25
−0.959	70.66	−1.309	93.16	−1.217	19.13	−0.759	3.46
−1.002	93.16	−1.461	116.66	−1.197	19.88	−0.789	3.93
−1.108	116.66	−1.528	122.41	−1.234	21.41	−0.839	4.40
−1.224	140.88	−1.645	140.88	−1.227	22.25		

Table 2
Dicloxacillin present in the suspension filtrate

T = 35°C		T = 40°C		T = 50°C		T = 60°C	
ln A	t	ln A	t	ln A	t	ln A	t
−1.580	0.00	−1.580	0.00	−1.619	0.00	−1.537	0.00
−1.589	6.16	−1.619	6.16	−1.635	1.00	−1.580	0.75
−1.666	21.25	−1.726	21.25	−1.693	2.00	−1.676	1.25
−1.772	44.33	−1.938	44.33	−1.732	3.06	−1.715	1.75
−1.864	51.83	−2.010	51.83	−2.171	18.33	−1.743	2.25
−1.945	70.66	−2.333	93.16	−2.216	19.13	−1.754	3.46
−1.995	93.16	−2.513	116.66	−2.207	19.88	−1.789	3.93
−2.120	116.66	−2.577	122.41	−2.263	21.41	−1.845	4.40
−2.254	140.88	−2.733	140.88	−2.254	22.25		

The shelf life determination

The zero order reaction constant (k_0) (saturated solution) is related to the corresponding first order rate constant (k_1) by means of the following equation:

$$k_0 = k_1 \times c_s$$

c_s being the solubility coefficient of the reactant substance at the temperature of interest [8].

Accordingly, the solubility coefficients of both penicillins were experimentally determined at 25°C. The values obtained for the ampicillin and dicloxacillin were 0.5354 and 0.1839% respectively.

Table 4 shows the experimental values of c_s and the calculated values of k_0, after relating these c_s values with the data shown in Table 3.

The shelf life ($t_{90\%}$) for a given drug containing several active substances is defined as the time interval necessary to degrade 10% of the least stable substance at 25°C.

In the present study the $t_{90\%}$ refers to the ampicillin and it is calculated as:

$$\frac{G}{M} 0.1 = k_0 \times t_{90\%}$$

where G is the quantity (g) of the sample in 1 l and M is the molecular weight of the ampicillin trihidrate.

Table 3

Temperature	k_1 (ampicillin)	k_1 (dicloxacillin)
60°C	$6.215 \times 10^{-2}\ h^{-1}$	$6.481 \times 10^{-2}\ h^{-1}$
50°C	$2.602 \times 10^{-2}\ h^{-1}$	$2.907 \times 10^{-2}\ h^{-1}$
40°C	$0.799 \times 10^{-2}\ h^{-1}$	$0.819 \times 10^{-2}\ h^{-1}$
35°C	$0.599 \times 10^{-2}\ h^{-1}$	$0.482 \times 10^{-2}\ h^{-1}$
25°C	$0.138 \times 10^{-2}\ h^{-1}$	$0.117 \times 10^{-2}\ h^{-1}$

Table 4

Penicillin	c_s (25°C)	k_0 (25°C)
Ampicillin	$0.0132\ mol\ l^{-1}$	$0.01826 \times 10^{-3}\ mol\ l^{-1}\ h^{-1}$
Dicloxacillin	$0.0036\ mol\ l^{-1}$	$0.00422 \times 10^{-3}\ mol\ l^{-1}\ h^{-1}$

Table 5

Ampicillin suspension without dicloxacillin. $T = 25°C$

$k_1 = 3.005 \times 10^{-3}\ h^{-1}$
$c_s = 0.0187\ mol\ l^{-1}$
$k_0 = 0.0562 \times 10^{-3}\ mol\ l^{-1}\ h^{-1}$

From this expression it is calculated that the shelf life for the suspension is 14 ± 2.72 days at 25°C.

Interestingly, the $t_{90\%}$ value of an ampicillin suspension without dicloxacillin (Table 5) was found to be only 4.6 days. The reason for this difference in behaviour is not understood and is the subject of continuing study.

References

[1] T. Berti, in *Le Associazioni Antibatteriche: Aspectti Farmacologi e Clinic*. Piccin Editore, Padova, Italia, 1973.
[2] H. B. Bundgaard and K. Ilver, *J. Pharm. Pharmac.* **24**, 790–794 (1972).
[3] M. Cervera, B. Nialet, A. Riera and J. de Bolos, *C.I.F. (2a. ép.), 374–379 (1982)*.
[4] *British Pharmacopoeia* (1980).
[5] HP 8451A. *Diode Array Spectrophotometer. Operator's Manual.*
[6] *Medicamentos de Actualidad VII*, p. 50 (1966).
[7] V. P. Spiridonov and A. A. Lopatkin, in Tratamiento Matemático de Datos Físico-Químicos. Mir. Moscú, (1973).
[8] E. Simo, V. Girona, J. de Bolos and M. Castillo, *An. Real Acad. Farm.* **49**, 463–472 (1983).

[*First received 23 October 1985; revised manuscript received 23 June 1986*]