Journal of Pharmaceutical and Biomedical Analysis 50 (2009) 576–579

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

A comparison of the stability of doxorubicin and daunorubicin in solid state

J. Cielecka-Piontek[a,*], A. Jelińska[a], M. Zając[a], M. Sobczak[a], A. Bartold[a], I. Oszczapowicz[b]

[a] Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Poznan University of Medical Sciences, Grunwaldzka 6, 60-780 Poznań, Poland
[b] Department of Modified Antibiotics, Institute of Biotechnology and Antibiotics, Starościńska 5, 02-515 Warszawa, Poland

ARTICLE INFO

Article history:
Received 10 July 2008
Received in revised form 7 December 2008
Accepted 11 December 2008
Available online 30 December 2008

Keywords:
Daunorubicin
Doxorubicin
Stability
Solid State
HPLC

ABSTRACT

The degradation of doxorubicin and daunorubcin in the solid state was studied using an HPLC method with UV detection (LiChrospher RP-18, 5 μm, 250 mm × 4 mm; mobile phase: acetonitrile-solution A 1:1, v/v (solution A: 2.88 g of laurisulfate sodium and 1.6 ml of phosphoric acid(V) in 1000 ml); flow rate – 1.4 ml min^{-1}; UV detection – 254 nm). The degradation of doxorubicin was a first-order reaction depending on the substrate concentration and daunorubicin degraded according to the kinetic model of autocatalysis. The dependence $\ln k_i = f(1/T)$ was described by the equations $\ln k_{DOX} = 40.0 \pm 15.6 - (19804 \pm 5682)\ (1/T)$ and $\ln k_{DAU} = 35.9 \pm 11.3 - (16581 \pm 3972)\ (1/T)$ at 76.4% RH. The dependence $\ln k_i = f(\mathrm{RH}\%)$ was described by the equations $\ln k_{DOX} = (8.80 \pm 3.60) \times 10^{-2}\ (\mathrm{RH}\%) - (21.50 \pm 2.57)$ and $\ln k_{DAU} = (6.63 \pm 1.22) \times 10^{-2}\ (\mathrm{RH}\%) - (13.35 \pm 1.68)$. The thermodynamic parameters (E_a, $\Delta H^{\neq a}$, $\Delta S^{\neq a}$) of the degradation of doxorubicin and daunorubicin were calculated. Although the degradation of doxorubicin was slower at increased temperature (353–373 K) and relative air humidity (50.9–90.0%), the differences between the influence of temperature and relative air humidity on the stability doxorubicin and of daunorubicin were not significant.

© 2008 Elsevier B.V. All rights reserved.

1. Introduction

Daunorubicin (DAU) and doxorubicin (DOX) are the most commonly used anthracycline anticancer antibiotics. Their molecules consist of a tetracyclic quinoid aglycone and an amino sugar–daunosamine, which are linked by a glycoside bond [1]. DAU and DOX may be susceptible to degradation, similarly to other glycosides. In order to ensure therapeutical safety it is important to determine the stability of DAU and DOX, particularly because of the fact that the aglycone and other products of degradation are mutagegenic and have no antitumor activity [2].

The photolytic degradation and stability of DOX in various infusion fluids have been studied [3,4]. The kinetics of hydrolysis of DOX at pH 0.4–2.1 has also been described. The degradation of DOX in 0.01–0.50 mg ml^{-1} hydrochloric acid was a first-order reaction, relative to the substrate concentration. Under such conditions the specific hydrogen ion catalytic rate constant and activation energy were 1.02 mol^{-1} h^{-1} and 92.0 kJ mol^{-1}, respectively [5].

Similar to DOX, DAU is degraded in aqueous solutions. The degradation kinetics of DAU at pH 0–14 at 50 °C has been studied. The influence of buffers, ionic strength and temperature on the degradation of DAU has been investigated. The activation energy of the degradation of DAU at pH 8.0 was 79 kJ mol^{-1} and at pH 1.5 was 114 kJ mol^{-1} [6].

The stability of DAU in influsion fluids has also been studied [4,7]. DAU stored in polypropylene syringes at 4 °C after diluting with water for injections was stable for at least 43 days [8]. Studies of the stability and compatibility of a mixture of etoposide, cytarabine and DAU have shown that all of them were stable in a 5% glucose solution, both when separate and mixed. The greatest stability was obtained by storing the mixture in the dark at room temperature [9].

The aim of our work was to evaluate and compare the stability of DOX and DAU in the solid state at increased relative air humidity and at various temperatures.

2. Experimental

2.1. Materials

DOX and DAU were synthesized at the Institute of Biotechnology and Antibiotics, Department of Modified Antibiotics, Warsaw, Poland. They were reddish powders, freely soluble in water and methanol.

Methyl p-hydroxybenzoate (Sigma–Aldrich Logistik GmbH) was used as an internal standard (IS). Sodium laurisulfate (A.C. reagent, Sigma–Aldrich Logistik GmbH) and all other chemicals were

* Corresponding author.
E-mail address: jpiontek@ump.edu.pl (J. Cielecka-Piontek).

0731-7085/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2008.12.029

obtained from Merck KGaA (Germany) and were of analytical or high-performance liquid chromatographic grade.

2.2. Equipment

Chromatographic separation and quantitative determination of DOX and DAU were performed by using a high-performance liquid chromatograph equipped with an LC-6A pump (Shimadzu, Kyoto, Japan), a UV–vis (SPD-6AV) detector (Shimadzu, Kyoto, Japan), a Rheodyne injector with a 50 μl loop. As the stationary phase a LiChrospher RP-18 column, 5 μm particle size, 250 mm × 4 mm (Merck, Darmstadt, Germany) was used. The UV detection was performed at 254 nm. The mobile phase consisted of acetonitrile:solution A (1:1,v/v); (solution A: 2.88 g of sodium laurisulfate and 1.6 ml of phosphoric acid(V) in 1000 ml). The flow rate was 1.4 ml min^{-1}.

2.3. Method validation

The method was validated according to the guidelines of the International Conference on Harmonization [10].

2.3.1. Selectivity

The selectivity of the HPLC method was examined for non-degraded samples and for degraded samples of DOX and DAU (In order to induce degradation DOX was stored at 363 K for 22 h and at 373 K for 77 h, 76.4% RH, whereas DAU was stored at 363 K for 25 h and at 373 K for 201 h, 76.4% RH).

2.3.2. Linearity

The calibration plots for $P_{DOX/DAU}/P_{IS} = f(c)$ were obtained in the concentration ranges $(2.50–22.50) \times 10^{-2}$ mg ml^{-1} (DOX) and $(5.00–70.0) \times 10^{-2}$ mg ml^{-1} (DAU), where P_{DOX}/P_{IS} and P_{DAU}/P_{IS} are the ratios of peak areas of DOX or DAU to methyl p-hydroxybenzoate (IS).

2.3.3. Precision

The precision of the assay was determined in relation to repeatability (intra-day). In order to evaluate the repeatability of the method eight samples of DOX and DAU were determined during the same day at three concentrations (DOX: 0.225 mg ml^{-1}, 0.200 mg ml^{-1}, 0.100 mg ml^{-1}; DAU: 0.40 mg ml^{-1}, 0.30 mg ml^{-1}, 0.20 mg ml^{-1}).

2.3.4. Limit of detection (LOD) and limit of quantitation (LOQ)

The LOD and LOQ were calculated from the regression equation $P_{DOX/DAU}/P_{IS} = f(c)$, where $LOD = 3.3S_y/a$ and $LOQ = 10S_y/a$. S_y denotes the standard deviation of the calibration curve and a is the slope of the corresponding calibration curve.

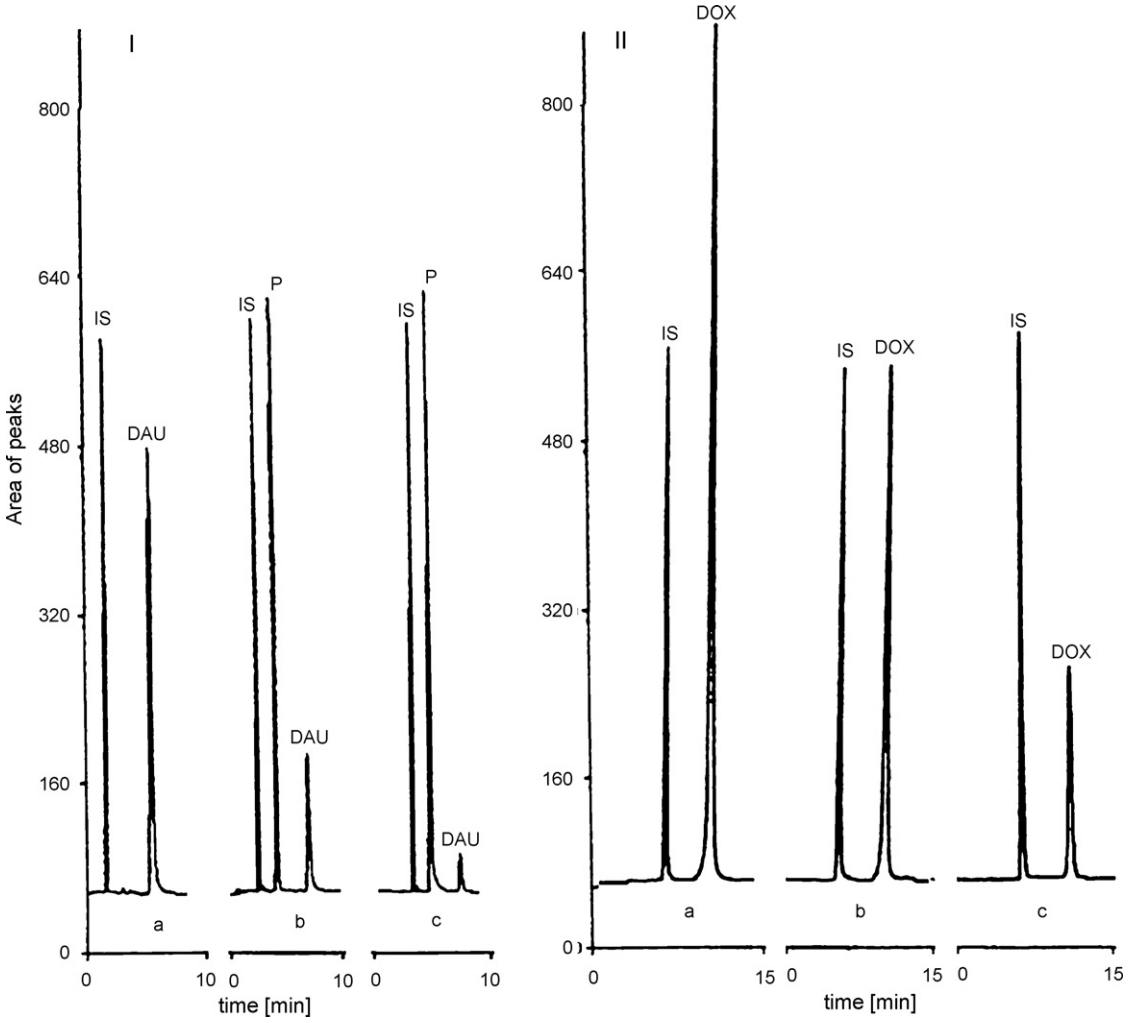

Fig. 1. HPLC chromatograms of daunorubicin (DAU) and doxorubicin (DOX), their degradation products (P) and internal standard (IS): Ia and IIa at $t = 0$ min; Ib after 22 h at 363 K, 76.4% RH and IIb after 77 h at 373 K, 76.4% RH; Ic after 25 h at 363 K, 76.4% and IIc after 201 h at 373 K, 76.4% RH.

2.4. Kinetic studies

For the forced aging test 5 mg samples of DOX and DAU were weighed into 5 ml vials. To evaluate the stability of DOX and DAU at increased air humidity the vials were placed in heat chambers at 333, 323, 343, 353, 363, and 373 K in desiccators containing saturated solutions of inorganic salts: sodium iodide (25.0% RH), sodium bromide (50.9% RH), potassium iodide (60.5% RH), sodium nitrate (66.5% RH), sodium chloride (76.4% RH) and zinc sulfate (90.0% RH) [11]. At specified time intervals, determined by the rate of degradation, the vials were removed, cooled to room temperature and their contents were dissolved in distilled water. The so-obtained solutions were quantitatively transferred into measuring flasks and diluted with water to 25.0 ml. To 1.0 ml of each of these solutions 2.0 ml of a 0.025 mg ml^{-1} of solution the internal standard (methyl p-hydroxybenzoate) was added.

3. Results and discussion

3.1. The validation of the HPLC method

The HPLC method, proposed by European Pharmacopoeia VI [12], after modification of the content of phosphoric acid(V) and the flow rate of the mobile phase, was proved to be suitable for the determination of DOX and DAU in studies of their stability in the solid state. The method was found selective for the determination of DOX and DAU (Fig. 1). As shown in the chromatograms, DOX and DAU formed symmetrical peaks, clearly separated from the peak of degradation products and that of methyl p-hydroxybenzoate (IS). The calibration plots were linear in the following concentration ranges: $(2.50–22.50) \times 10^{-2}$ mg ml^{-1} for DOX ($n = 9$, $r = 0.9999$) and $(5.0–70.0) \times 10^{-2}$ ($n = 14$, $r = 0.9994$) for DAU. The calibration curves are described by the equation $y = ac$ (the values b, calculated from the equation $y = ac + b$, were not significant); $y = (16.70 \pm 0.25) \times c$ for DOX and $y = (2.52 \pm 0.51) \times c$ for DAU. The parameters of regression were calculated for $f = n - 2$ degrees of freedom with $\alpha = 0.05$. The method had good intra-day repeatability: the RSD for DOX was 2.30–3.91% and for DAU 0.12–1.21%. The LOD for DOX was 4.10×10^{-3} mg ml^{-1} and for DAU was 2.50×10^{-2} mg ml^{-1}. The LOQ were 1.24×10^{-2} mg ml^{-1} and 5.00×10^{-2} mg ml^{-1} for DOX and DAU, respectively.

3.2. The kinetic parameters of the degradation of DOX and DAU

The degradation of DOX during incubation at increased relative air humidity (25.0–90.0%) and temperature (353–373 K) was a first-order reaction described by the following equation:

$$\ln P_{DOX} = \ln P_{DOX0} - k_{obs} \times t$$

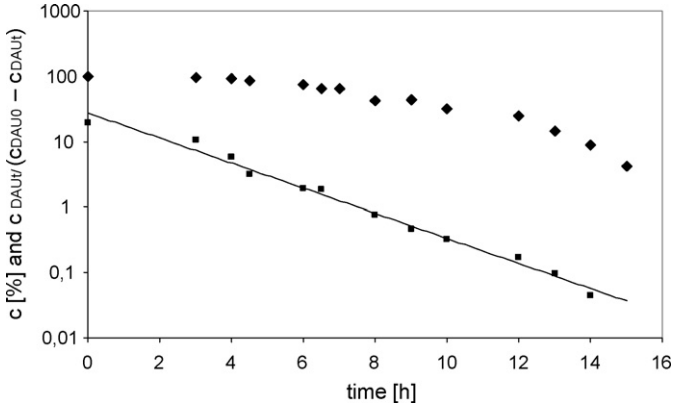

Fig. 2. Semilogarithmic plots of $c_{DAU}[\%] = f(t)$ (♦) and $c_{DAUt}/(c_{DAU0} - c_{DAUt}) = f(t)$ (■) for the degradation of daunorubicin at 90.0% RH in solid state at 363 K; where: c_{DAU} concentration of DAU, c_{DAU0} – concentration of DAU at 0 min; C_{DAUt} – concentration of DAU at t, min.

During the degradation of DOX the ratio P_{DOX}/P_{IS} decreased in the time interval $t_0 \rightarrow t_\infty$ from $(P_{DOX}/P_{IS})_{max}$ to $(P_{DOX}/P_{IS})_0$.

The degradation of DAU during incubation at increased relative air humidity (50.9–90.0%) and temperature (333–373 K) was an autocatalytic reaction described by the following equation:

$$\ln c_{DAUt}/(c_{DAU0} - c_{DAUt}) = -k_{obs} \times t$$

The changes in the concentration of DAU were not linear because in this reaction model an induction phase with a very small substrate loss is initially observed, which is followed by an acceleration phase that involves a rapid substrate degradation. The dependence $\ln c_{DAUt}/(c_{DAU0} - c_{DAUt}) = f(t)$ is a straight-line relationship (Fig. 2). The presence of a hydroxyl group in a DOX molecule changes the kinetic mechanism of its degradation. In contrast to DAU, the degradation of DOX is a first-order reaction. It is a significant change because in this degradation model it is easier to predict the time period during which DOX demonstrates the desired stability.

The observed rate constants are equal to the slopes of the plots $\ln(P_{DOX}/P_{IS}) = f(t)$ for DOX and $\ln c_{DAUt}/(c_{DAU0} - c_{DAUt}) = f(t)$ for DAU, with a negative sign ($-k_{obs}$), and are presented in Tables 1 and 2. The following statistical parameters of the equation $y = ax + b$ were calculated by using the least squares method: $a \pm \Delta a$, $b \pm \Delta b$, standard deviations S_a, S_b, S_y and the coefficient of linear correlation r. The values Δa and Δb were calculated for $f = n - 2$ degrees of freedom and $\alpha = 0.05$.

Table 1
Kinetic and thermodynamic parameters of the degradation of DOX and DAU in the solid state at 76.4% RH.

Temperature [K]	$(k \pm \Delta k)$ [s^{-1}]	Statistical evaluation $\ln k = f(1/T)$	Thermodynamic parameters
DOX			
353	$(1.20 \pm 0.10) \times 10^{-7}$	$a = -19804 \pm 5682$	$E_a = 164 \pm 47$ (kJ mol^{-1})
358	$(1.87 \pm 0.14) \times 10^{-7}$	$S_a = 1786$	$\Delta H^{\neq a} = 162 \pm 50$ (kJ mol^{-1})
363	$(4.82 \pm 0.39) \times 10^{-7}$	$b = 40.0 \pm 15.6$ $S_b = 4.92$	$\Delta S^{\neq a} = -87 \pm 114$ (J K^{-1} mol^{-1})
368	$(1.18 \pm 0.13) \times 10^{-6}$	$r = 0.9889$ $S_y = 0.2090$	
373	$(1.90 \pm 0.14) \times 10^{-6}$		
DAU			
333	$(7.12 \pm 0.81) \times 10^{-7}$	$a = -16581 \pm 3972$	$E_a = 138 \pm 33$ (kJ mol^{-1})
343	$(5.32 \pm 0.71) \times 10^{-6}$	$S_a = 1248$	$\Delta H^{\neq a} = 135 \pm 35$ (kJ mol^{-1})
353	$(2.26 \pm 0.38) \times 10^{-5}$	$b = 35.94 \pm 11.3$ $S_b = 3.5$	$\Delta S^{\neq a} = -149 \pm 203$ (J K^{-1} mol^{-1})
363	$(5.25 \pm 1.95) \times 10^{-5}$	$r = 0.9916$ $S_y = 0.3178$	
373	$(1.75 \pm 0.02) \times 10^{-4}$		

$\Delta k = S_a t_\alpha f E_a$,activation energy; $\Delta H^{\neq}$, enthalpy; $\Delta S^{\neq}$, entropy; $E_a = -aR$; $\Delta H^{\neq} = E_a - TR$; $\Delta S^{\neq} = R\,(\ln A\ \ln(k_b T)/h$ where: k_B, Boltzmann's constant (1.3807×10^{-23} J K^{-1}); h, Planck's constant (6.626 10^{-34} J s^{-1}); R, universal gas constant (8.314 K^{-1} mol^{-1}), T, temperature in K ($t + 273$ K); a, vectorial coefficient of the Arrhenius; A, frequency coefficient a calculated for 298 K.

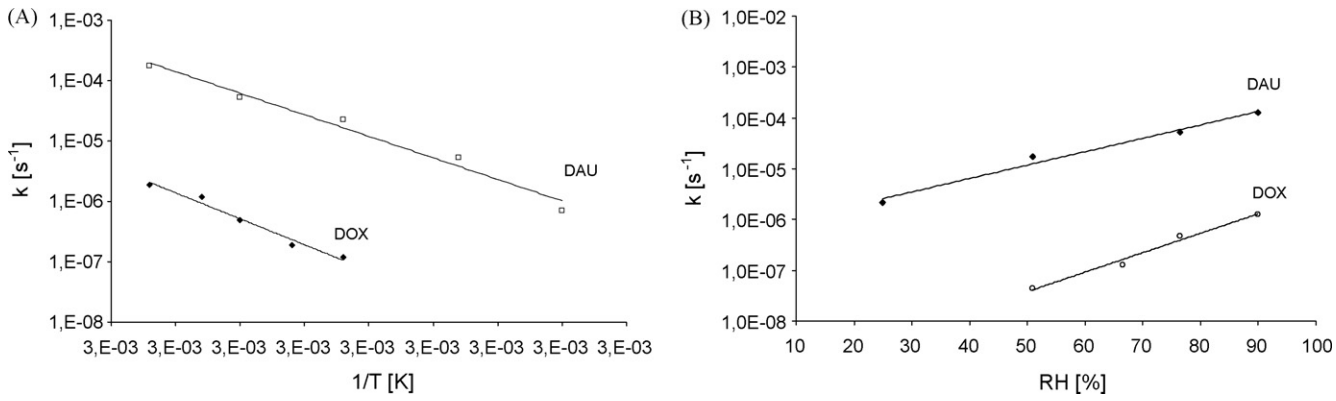

Fig. 3. The semilogarithmic relationship $k_i = f(1/T)$ for the degradation of DOX and DAU at 76.4% RH (A) and the relationship $\ln k_i = f(\mathrm{RH\%})$ for the DOX and DAU in solid state, at 363 K (B).

Table 2
The effect of relative air humidity on the stability of DOX and DAU at 363 K.

Relative air humidity (%)	$(k \pm \Delta k)$ [s^{-1}]	Statistical evaluation $\ln k = f(\mathrm{RH\%})$
DOX		
50.9	$(4.48 \pm 0.71) \times 10^{-8}$	$a = (8.80 \pm 3.60) \times 10^{-2}$ $S_a = 8.28 \times 10^{-3}$
66.5	$(1.28 \pm 0.05) \times 10^{-7}$	$b = -21.50 \pm 2.57$ $S_b = 0.599$
76.4	$(4.82 \pm 0.39) \times 10^{-7}$	$r = 0.9910$ $S_y = 0.236$
90.0	$(1.25 \pm 0.10) \times 10^{-6}$	
DAU		
25.0	$(2.19 \pm 0.66) \times 10^{-6}$	$a = (6.63 \pm 1.22) \times 10^{-2}$ $S_a = 6.08 \times 10^{-3}$
50.9	$(1.73 \pm 0.31) \times 10^{-5}$	$b = -13.35 \pm 1.68$ $S_b = 0.390$
76.4	$(5.25 \pm 1.95) \times 10^{-5}$	$r = 0.9917$ $S_y = 0.110$
90.0	$(1.29 \pm 0.15) \times 10^{-4}$	

3.3. The effect of temperature on the degradation of DOX and DAU

Based on the Arrhenius relationship, $\ln k_i = \ln A - E_a/RT$, the linear plots of $\ln k = f(1/T)$ were used to calculate the energy of activation (E_a) and the pre-exponential coefficient (A) for the degradation of DOX and DAU (Table 1). The values of activation energy did not demonstrate statistically significant differences and ranged from 138 to 164 kJ mol^{-1}. The value of entropy of the degradation of DAU and DOX was negative, which may indicate the bimolecular character of degradation.

At increased relative air humidity (76.4% RH) and in the temperature range 333–373 K DOX was more stable than DAU (Fig. 3A). For the evaluation of the influence of temperature on the stability of DOX and DAU in the solid state the slopes of the plots $\ln k_i = f(1/T)$ were compared by using the parallelism test. At increased relative humidity the differences between the influence of temperature on the stability of DOX and DAU were not statistically significant.

3.4. The influence of relative air humidity on the stability of DOX and DAU

The influence of relative air humidity on the stability of DOX and DAU is described by the following equations:

$$\ln\ k_{DOX} = (8.80 \pm 3.60)10^{-2}(RH\%) - (21.50 \pm 2.57)$$

$$\ln\ k_{DAU} = (6.63 \pm 1.22)10^{-2}(RH\%) - (13.35 \pm 1.68)$$

The slopes a express the effect of relative air humidity on the stability of DOX and DAU in the solid state and the value 10^b denotes their stability at 0% RH (Table 2).

The observed rate constants of DOX and DAU obtained at increased relative air humidity (50.9–90.0%) were statistically different which indicated the greater stability of DOX. The influence of relative air humidity on the degradation of DOX and DAU was statistically significant, although the parallelism test proved, after comparing the plots $\ln k_1 = f(\mathrm{RH\%})$, that the influence of relative air humidity on the stability of DOX and that of DAU was similar (Fig. 3B).

4. Conclusion

The study has demonstrated that the differences in the chemical structures of DOX and DAU influence their stability and the kinetic mechanism of their degradation. It is possible that the first-order reaction of degradation and greater stability of DOX at increased temperature and relative air humidity are caused by the presence of a hydroxyl group in its molecule.

References

[1] F. Arcamone, A.G. Franceshi, S. Pencom, S. Selva, Tetrahedron Lett. (1969) 1007–1010.
[2] C. Cordaro, T. Cebula, J. Ramsey, FDA/PRT Semiannual Report, AIC-R-29, 1980 (4).
[3] N. Tavoloni, A.M. Guanino, P.D. Berk, J. Pharm. Parmacol. 32 (1980) 860–862.
[4] G.K. Poochikian, J.C. Cradock, K.P. Flora, Am. J. Hosp. Phar. 38 (1981) 483–486.
[5] K. Wassermann, H. Bundgaard, Inter. J. Pharm. 14 (1983) 73–78.
[6] J.H. Beijnen, O.A. van der Houwen, M.C. Voskuilen, W.J. Underberg, Inter. J. Pharm. 31 (1986) 75–82.
[7] J. Karlsen, H. Thonnensen, O. Resberg, S. Horne, T.J. Skobba, Norw. Pharm. Act. 45 (1983) 61–67.
[8] M.J. Wood, W.J. Irwin, D.K. Scott, J. Clin. Pharm. Therap. 15 (1990) 279–289.
[9] R. Chevrier, V. Sautou, V. Pinon, F. Demeocq, J. Chopineau, Pharm. Act. Hel. 70 (1995) 141–148.
[10] Validation of analytical procedures, in: Proceeding of the International Conference of Harmonisation (ICH), Commission of the European Comminities, 1996.
[11] E. Pawełczyk, T. Herman, The Fundamentals of Stability of Drugs (in Poland), PZWL, Warsaw, 1982.
[12] European Pharmacopeia VI.