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Short communication

Stability of epidoxorubicin in solid state

Agnieszka Sobczak[a,*], Anna Jelińska[a], Monika Leśniewska[a], Artur Firlej[a], Irena Oszczapowicz[b]

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

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ABSTRACT

The influence of temperature and relative air humidity on the stability of epidoxorubicin hydrochloride (EP) was investigated. The degradation of the substance studied was determined: (a) in dry air at 393 K, (b) at relative air humidity ~76% at 333 K, 343 K, 353 K, 363 K and 373 K, (c) in the relative air humidity range 50–90% at 363 K. The degradation of EP in the atmosphere of increased relative air humidity was a first-order reaction relative to substance concentration and in dry, hot air (RH 0%; 393 K) is a reversible first-order reaction relative to substance concentration. The dependences $\ln k = f(1/T)$ and $\ln k = f(\mathrm{RH}\%)$ were described by the equation: $\ln k = (35.1 \pm 10.9) - (16{,}250 \pm 3823)(1/T)$ and $\ln k = (3.79 \pm 3.34) \times 10^{-2}(\mathrm{RH}\%) - (12.9 \pm 2.4)$, respectively. The kinetic and thermodynamic parameters of EP degradation were calculated. The parameters of separation were following: LiChrospher RP-18 column, 5 μm, 250 mm × 4 mm; mobile phase: the mixture of equal volume of acetonitrile and the solution containing 2.88 g l^{-1} of sodium laurisulfate and 2.25 ml l^{-1} of phosphoric acid (V) 85%; flow rate: 1.0 ml min^{-1}; UV detection – 254 nm.

1. Introduction

Stability can be defined as the capacity of the drug substance or drug product to sustain its identity, strength, quality and purity throughout the retest or expiration period [1]. Stability testing of drug substances or products investigates the changes of the quality of a drug product under the influence of environment factors such as temperature, humidity and light.

The International Conference on Harmonization (ICH) Guidelines Q1A (R2) require the use of validated stability-indicating analytical procedures for stability testing of a drug substance and product [2]. The results of these studies allow to establish a shelf life for the drug product and to recommend storage conditions.

Epidoxorubicin hydrochloride belongs to the second generation of anthracycline antibiotics. It is produced by *Streptomyces* species and is characterized by a wide spectrum of anticancer activity. Its molecule consists of a tetracyclic quinoid aglycone connected by a glycoside bond with an amino sugar – L-acosamine. Epidoxorubicin exhibits a different stereochemistry of the hydroxyl group at C-4′ compared to the parent compound doxorubicin. This structure is responsible for its lower cardiotoxicity and because of this epidoxorubicin can be used in higher doses [3–5]. EP degradation in aqueous solutions [6] and photostability [7,8] were reported previously. The process of degradation in aqueous solution occurred according to the first-order reaction relative to substrate concentration. In sodium hydroxide solutions (0.05 mol l^{-1}) and hydrochloric acid (0.05 mol l^{-1}) the positive salts effect was observed [6]. The rate of photolytic degradation is inversely proportional to the concentration of drug and increases with higher pH of the solvent. But in the case of solutions with concentration ≥500 μg ml^{-1}, which are used in therapy, the protection from light is not necessary [7]. Moreover, EP can be stored in 0.9% NaCl in minibags from PVC for 20 days at 25 °C and longer at 4 °C. It is recommended to store EP medical preparations in polypropylene packing to prevent adsorption [8].

The aim of this study was to develop and validate an RP-HPLC method that allows to determine the effect of temperature and relative air humidity on the investigated substance in a solid state. This research results from the fact that epidoxorubicin is produced as powder for injections.

2. Experimental

2.1. Materials and reagents

Epidoxorubicin hydrochloride was synthesized from the Institute of Biotechnology and Antibiotics, Warszawa, Poland; sodium laurisulfate A.C. reagent, Sigma-Aldrich Logistik GmbH; methyl 4-hydroxybenzoate, POCh, Gliwice, Poland. All other chemicals and solvents were of analytical or high-performance liquid chromatographic grade.

* Corresponding author.
E-mail address: asobczak@ump.edu.pl (A. Sobczak).

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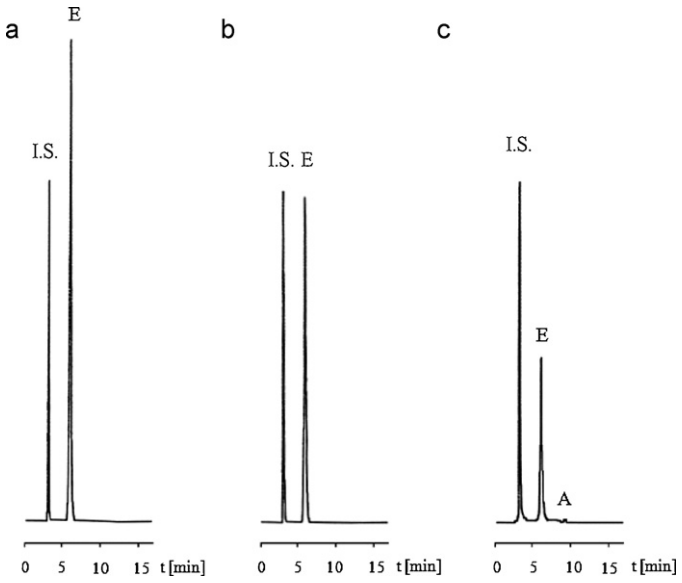

Fig. 1. HPLC chromatograms of EP, its degradation product (A) and the internal standard (I.S.) after incubation at 333 K (~76.4% RH): (a) $t = 0$ min; (b) 169.5 h; (c) 339 h.

2.2. Equipment

The analytical system was composed of a Rheodyne Berkeley 7120 with a noose of 50 μl, a isocratic pump model LC-61 Shimadzu and an SPD-6AV UV/VIS detector, which was set at 254 nm. A Merck analytical column LiChrospher RP-18, 250 mm × 4 mm (5 μm) was used as the stationary phase. The mobile phase, the mixture of equal volume of acetonitrile and the solution containing 2.88 g l^{-1} of sodium laurisulfate and 2.25 ml l^{-1} of phosphoric acid (V) 85% was used at flow rate 1 ml min^{-1}. The internal standard was methyl 4-hydroxybenzoate (I.S.) in a mixture of ethanol and water (1:1) at a concentration of 0.025 mg ml^{-1}.

2.3. Method validation

The HPLC method was validated according to *the International Conference on Harmonization Guidelines* with regard to selectivity, linearity, precision, limits of detection and quantitation. The method was selective for EP and the internal standard. As shown in the chromatograms (Fig. 1), EP formed symmetrical peaks, clearly separated from the peak of methyl 4-hydroxybenzoate (IS). Peak areas $P_i/P_{I.S.}$ (P and $P_{I.S.}$ – areas of EP and I.S.) and concentrations of EP were subjected to the least square linear regression analysis to calculate the calibration equations and correlation coefficients. Each sample was prepared in triplicate and ten different concentrations were used for the linearity studies. In the range from 0.0150 mg ml^{-1} to 0.1500 mg ml^{-1} (15–150% of the nominal concentration of the substrate used in the stability study) calibration curve was described by the equation: $y = (25.5 \pm 0.7) \cdot x + (10.1 \pm 6.3) \times 10^{-2}$; $r = 0.9995$. These results demonstrate good correlation between the peak area and analyte concentration. The method had adequate repeatability because the relative standard deviation (RSD) for six repeated assays of samples was less than 1.0% for three different concentrations of EP: 0.66% for 0.05 mg ml^{-1}, 0.10 mg ml^{-1} and 0.79% for 0.15 mg ml^{-1}. The limits of detection (LOD = 5.06×10^{-3} mg ml^{-1}) and quantitation (LOQ = 1.53×10^{-2} mg ml^{-1}) were calculated from the formulas LOD = $3.3S_y/a$ and LOQ = $10S_y/a$, where S_y is the standard deviation of the blank signal and a is the slope of the corresponding calibration curve.

2.4. The kinetic parameters of epidoxorubicin hydrochloride degradation

Within the stress test, 5 mg samples of EP were weighed into 2 ml vials and exposed to the following conditions:

- in the atmosphere of relative air humidity ~76.4% at 333 K, 343 K, 353 K, 363 K, 373 K,
- at 363 K, at relative air humidity 50.9%, 66.5%, 76.4%, 90.0%,
- at relative air humidity 0% at 393 K.

In order to determine the stability of EP in dry air, the vials containing the studied preparation were immersed in sand baths, in heat chambers adjusted to 393 K.

The appropriate values of relative air humidity were obtained by using desiccators containing saturated solutions of suitable inorganic salts: 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).

At appropriate time intervals, determined by the rate of degradation, the vials with 5 mg of EP were removed from the sand bath or from the desiccators placed in heat chambers, cooled to room temperature and their contents were dissolved in the mobile phase. The so-obtained solutions were quantitatively transferred into measuring flasks and diluted with mobile phase to 25.0 ml. To 1.0 ml of each of these solutions 1.0 ml of a 0.025 mg ml^{-1} of the internal standard solution was added. 50 μl of these solutions were injected onto the column. Because of the anthracyclines are photolabile all samples with EP were protected from light.

3. Results and discussion

The RP-HPLC method was developed for quantitative analysis of EP in the solid state.

The degradation of epidoxorubicin hydrochloride in the solid state:

- in the atmosphere of relative air humidity ~76.4% at 333 K, 343 K, 353 K, 363 K, 373 K and at 363 K, at relative air humidity 50.9%, 66.5%, 76.4%, 90.0%, was a first-order reaction depending on the substrate concentration described by the following equation (Fig. 2):

$$\ln\left(\frac{P_t}{P_{I.S.}}\right) = \ln\left(\frac{P_0}{P_{I.S.}}\right) - k_{obs} \cdot t$$

- at relative air humidity 0% at 393 K was the reversible first-order reaction depending on the substrate concentration according to the equation (Fig. 3):

$$\ln(P - P_\infty) = \ln(P_0 - P_\infty) - k_{obs} \cdot t$$

where P_0, P_t – peak areas of EP at time zero and time t, respectively; $P_{I.S.}$ – peak area of I.S.; k_{obs} – observed first-order reaction rate constant.

The semilogarithmic plots $P = f(t)$ and $P - P_\infty = f(t)$ were linear and their slopes equal to the rate constants of the reaction with the negative sign ($-k$). For these plots the least squares method was used to calculate the parameters of the equation $y = ax + b$, $a \pm \Delta a$, $b \pm \Delta b$, standard errors S_a, S_b, S_y and the correlation coefficient r. The values $\pm\Delta a$, $\pm\Delta b$ were calculated for $f = n - 2$ degree of freedom and $\alpha = 0.05$.

The influence of temperature on the rate constant of EP degradation was estimated basing on the Arrhenius relationship:

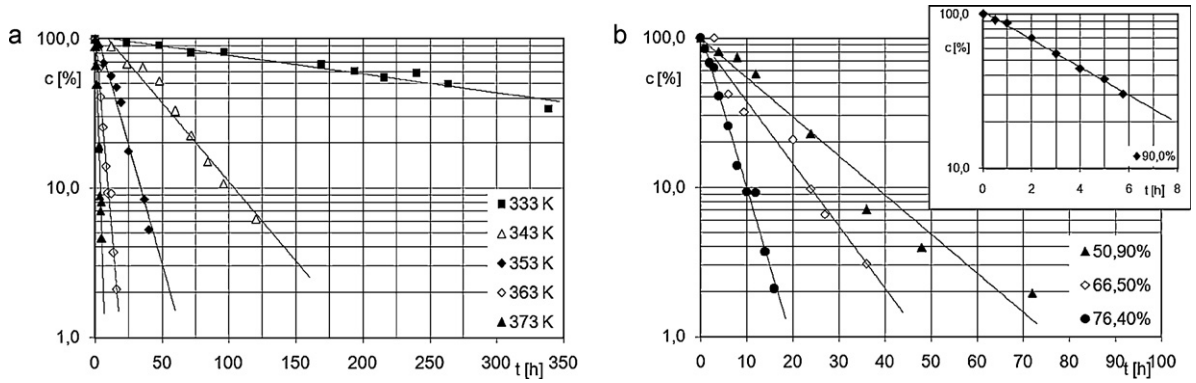

Fig. 2. Semilogarithmic plots $c = f(t)$ of the degradation of epidoxorubicin hydro-chloride: (a) at different temperatures (~76.4% RH); (b) at various humidities at 363 K.

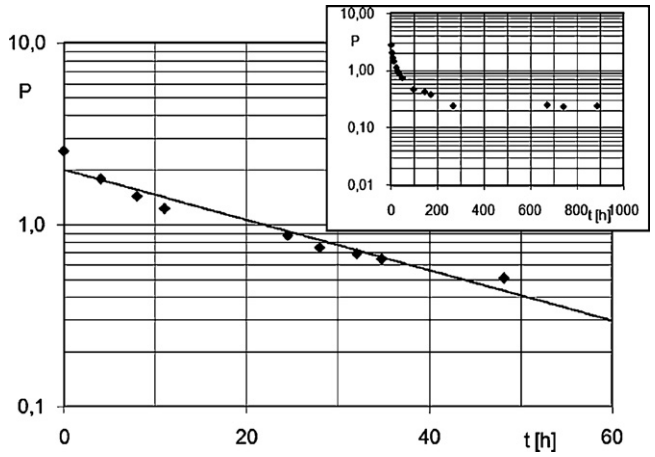

Fig. 3. Semilogarithmic plots $c = f(t)$ of the degradation of epidoxorubicin hydrochloride at 393 K at RH 0%.

$\ln k_i = \ln A - E_a/(\mathrm{RT})$. From the linear plot $\ln k_i = f(1/T)$, the pre-exponential coefficient (A) was calculated and was used to calculate energy (E_a), enthalpy ($\Delta H^{\neq}$) and entropy ($\Delta S^{\neq}$) (Table 1, Fig. 4).

Based on the equation $\ln k = \ln k + a$ RH% the influence of relative air humidity was described. The slope a of plot $\ln k = f(\mathrm{RH\%})$ expressed the effect of relative air humidity on the stability of EP in the solid state (Fig. 4).

Other compounds like doxorubicin (DOX) and daunorubicin (DAU) have the structure similar to epidoxorubicin and their stability in the solid state was described previously (Fig. 5) [9]. Their kinetic degradation was estimated by using the HPLC method, which differs from the above described method the amount of phosphoric acid (V) 85% and flow rate. Also the ratio of EP solution and the internal standard solution in injected samples was different. This research demonstrates that the degradation of doxorubicin in the solid state is a first-order reaction depending on the substrate concentration described by the same equation as for EP. However, the degradation of daunorubicin in the same conditions is an autocatalytic reaction described by the following equation: $\ln c_{\mathrm{DAUt}}/(c_{\mathrm{DAU0}} - c_{\mathrm{DAUt}}) = -k_{\mathrm{obs}} \cdot \mathrm{t}$.

Table 1
The first-order rate constants and thermodynamic parameters of the reaction of epidoxorubicin hydrochloride at various temperatures (76.4% RH).

Lp.	T (K)	$(k \pm \Delta k) \times 10^7$ (s^{-1})	Parameters of regression $\ln k_i = f(1/T)$	Thermodynamic parameters
1	333	$(8.02 \pm 1.28) \times 10^{-7}$	$a = -16{,}250 \pm 3823$	$E_a = 135 \pm 32$
2	343	$(6.79 \pm 0.93) \times 10^{-6}$	$S_a = 1202$	(kJ mol^{-1})
3	353	$(2.02 \pm 0.30) \times 10^{-5}$	$b = 35.1 \pm 10.9$	$\Delta H^{\neq} = 133 \pm 32$
4	363	$(6.61 \pm 0.59) \times 10^{-5}$	$S_b = 3.41$	(kJ mol^{-1})[a]
5	373	$(1.80 \pm 0.24) \times 10^{-4}$	$S_y = 0.309$	$\Delta S^{\neq} = 46.8 \pm 90.2$
			$r = -0.992$	(J K^{-1} mol^{-1})[a]

n, Number of experiments; $\Delta k = S_a\ t_{\alpha f}$; E_a, activation energy; $\Delta H^{\neq}$, enthalpy; $\Delta S^{\neq}$, entropy; $E_a = -aR$ (J mol^{-1}); $\Delta H^{\neq} = E_a - \mathrm{RT}$ (J mol^{-1}); $\Delta S^{\neq} = R(\ln A - \ln(k_B T)/h)$ (J K^{-1} mol^{-1}), where: k_B stands for the Boltzman constant (1.3807×10^{-23} J K^{-1}); h, Planck constant (6.6256×10^{-24} J s); R, universal gas constant (8.314 J K^{-1} mol^{-1}); T, temperature in K; a, vectorial coefficient of the Arrhenius relationship; and A, stands for the frequency coefficient.

[a] Calculated for 298 K.

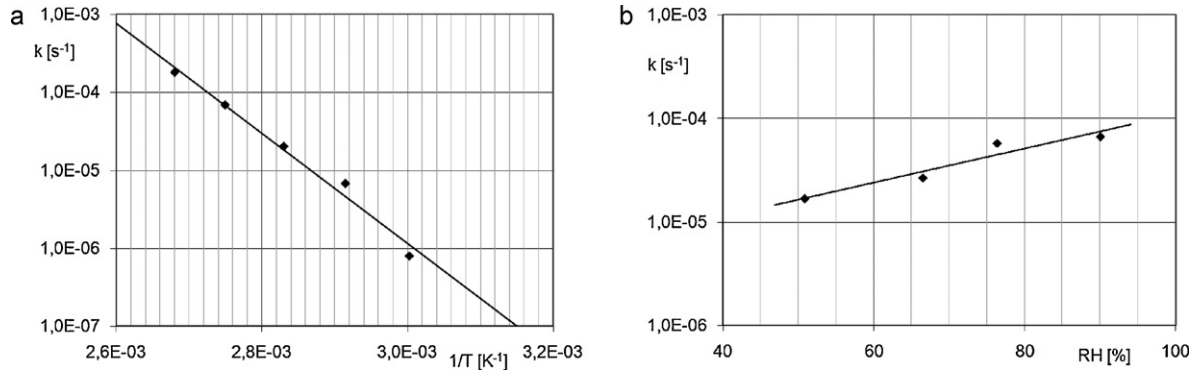

Fig. 4. Semilogarithmic relationship $k = f(1/T)$ for the degradation of EP at 76.4% (a) and relationship $\ln k = f(\mathrm{RH\%})$ for EP in solid state (b).

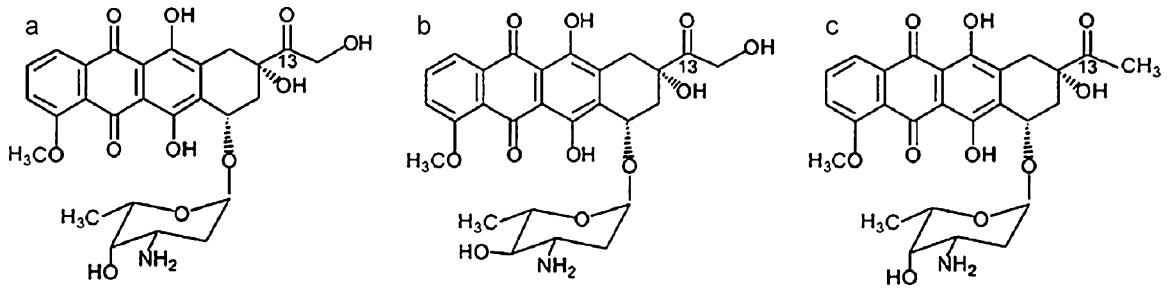

Fig. 5. The chemical structure of doxorubicin (a), epidoxorubicin (b) and daunorubicin (c).

At increased relative air humidity and in the temperature range 333–373 K, EP shows the least stability of the three compounds (EP, DAU and DOX). It can also be observed that EP stability in the solid state depends on relative air humidity to a lesser degree than in DOX and DAU.

In the conditions used to test the three compounds DOX proved to have the highest activation energy ($E_a = 164 \pm 47\ \mathrm{kJ\,mol^{-1}}$) and thus the highest stability (EP: $E_a = 135 \pm 32$; DAU: $E_a = 138 \pm 33\ \mathrm{kJ\,mol^{-1}}$) [9].

This research has demonstrated that the differs in the stereochemistry of the hydroxyl group at C-4′ in the structure of EP and DOX does not influence on the kinetic mechanism of their degradation, but only on the rate of degradation.

References

[1] FDA, Draft Guidance for Industry Stability Testing of Drug Substances and Drug Products, FDA, Rockville, 1998.
[2] ICH, International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, ICH Harmonized Tripartite Guideline: Stability Testing of New Drug Substances and Products Q1A (R2), Step 5, ICH, Geneva, 2003.
[3] F. Arcamone, et al., New developments in antitumor anthracyclines, Pharmacol. Ther. 76 (1997) 117–124.
[4] J. Bouma, et al., Anthracycline antitumor agents. A review of physicochemical, analytical and stability properties, Pharm. Weekbl. (Sci.) 8 (1986) 109–133.
[5] R. Zucchi, R. Danesi, Cardiac toxicity of antineoplastic anthracyclines, Curr. Med. Chem. Anticancer Agents 3 (2003) 151–171.
[6] A. Jelińska, M. Zając, K. Krzysztoń, The use of HPLC for determinating the stability of epirubicin and the influence of ionic strength on the stability of epirubicin, Ann. UMCS Lublin – Polonia 19 (2006) 45–50.
[7] M. Wood, W. Irwin, D. Scott, Photodegradation of doxorubicin, daunorubicin and epirubicin measured by high-performance liquid chromatography, J. Clin. Pharm. Ther. 15 (1990) 291–300.
[8] M. Wood, W. Irwin, D. Scott, Stability of doxorubicin, daunorubicin and epirubicin in plastic syringes and minibags, J. Clin. Pharm. Ther. 15 (1990) 279–289.
[9] J. Cielecka–Piontek, A. Jelińska, M. Zając, A comparison of the stability of doxorubicin and daunorubicin in solid state, J. Pharm. Biomed. Anal. 50 (2009) 576–579.