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The use of UV-VIS spectroscopy for determining the photostability of epirubicin solutions

Zastosowanie spektroskopii UV-VIS do oceny fototrwałości roztworów epirubicyny

Anthracyclines are very effective anticancer drugs [4, 6] but their clinical use [8] is limited by their toxic effect on healthy tissue (cardiomyopathy) [9] and the drug resistance of tumor cells. Epirubicin is a second-generation antracycline antibiotic used in the therapy of lymphomas, sarcomas and a wide range of carcinomas [8]. The stability of antracyclines is affected by many factors including storage temperature, pH of the vehicle and the light. The stability of epirubicine in aqueous solutions has been studied [2, 3, 5, 7]. Information of the photostability of epirubicine involves the influence of fluorescent light and sunlight [11].

The aim of this study was to determine the photostability of epirubicin according to ICH Guidelines. In order to determine the observed rate constants a UV-VIS spectroscopy method was used.

EXPERIMENTAL DESIGN

MATERIAL AND METHODS

Epirubicin was obtained from the Institute of Biotechnology and Antibiotics, Warsaw, Poland. All solvents and chemicals were of analytical grade. Quantitative analysis was preformed by using a double-beam spectrophotometer (UV-VIS 160 A, *Shimadzu*) with *PC 160 Plus* software. Detection was performed at 480 nm.

Photodegradation was carried out following the recommendations of the International Conference on Harmonization – version 1. A high-pressure mercury lamp (HBO-50) served as radiation source. An interference filter and a Wood's filter were used to isolate the wavelength 365 nm and 510 nm regions. Solutions of epirubicin were irradiated in a cylindrical, quartz cell (volume = 2.8 mL, l = 1 cm). A sample cell, protected against light by aluminium foil, was used as a dark control [1, 10]. The intensity of irradiation from the mercury lamp was measured by 2% *W/V* aqueous solution of quinine monohydrochloride dehydrate – a chemical actinometer (option 2 of the ICH Guidelines).

VALIDATION OF THE METHOD

The method was validated according to the International Conference on Harmonization Guidelines for validation of analytical procedures.

Linearity. Calibration curves for spectroscopic analysis were determined by linear regression. The linearity between A (absorbance) and the concentration of epirubicine in the water solutions ranging 0.012–0.06 mg mL^{-1} were evaluated. Linearity was also examined for three consecutive days in solutions of the same concentration prepared from the stock solution.

Precision. The precision of the assay was determined as repeatability (intra-day) and intermediate precision (inter-day). In order to evaluate the repeatability of the method six individual samples of four different concentrations (0.020, 0.024, 0.032 and 0.040 mg mL^{-1} epirubicin) were prepared and analyzed on the same day. The intermediate precision (inter-day) was studied by comparing the assays from two different days at epirubicine concentration of 0.020, 0.024, 0.032 and 0.040 mg mL^{-1}.

The limits of detection (LOD) and quantitation (LOQ) were calculated from the formulas LOD = 3.3 SD/*a* and LOQ = 10 SD/*a*, where SD is standard deviation and *a* is the slope of the corresponding calibration curve.

KINETIC MEASUREMENTS

During preparation studies all solutions of epirubicine were protected from light.

The initial photostability study of epirubicin in solutions was performed after expositing it to cool white fluorescent and near ultraviolet lamp radiation, according to the ICH Q1B Guidelines. To ensure the specified light exposure the samples was exposed side-by-side with a validated chemical actinometric system. In the second part of the study the influence of radiation at λ_{max} = 365 nm and λ_{max} = 510 nm at room temperature was investigated. The initial concentration of epirubicin was 0.032 mg mL^{-1}. At specified time intervals, determined by the rate of degradation, the absorbance of the solution was measured.

Microsoft Excel 2007 was used for the calculation of regression parameters.

RESULTS AND DISCUSSION

Photostability studies of epirubicin were performed according to the ICH recommendations, for studies of photochemical stability of drug substance and products. As shown in Fig. 1, the spectrum of epirubicin shows four maxima at 232 nm, 254 nm 291 nm and 480 nm. Exposure of epirubicine to light produced changes in the UV-VIS spectrum that involved a decrease in the intensity of the band at λ_{max} = 480 nm. Changes in epirubicin concentration under experimental conditions were measured using the UV-VIS method described in this study. The method was validated with respect to linearity, precision, detection and quantitation limits. The assays exhibited linearity between the absorbance and concentration of epirubicin over the range 0.012 – 0.060 mg mL^{-1} (Fig. 2). The equation for the calibration curve is $y = (19.78 \pm 0.44)x$, n = 10, r = 0,9995. The *b* value calculated from equation $y = ax + b$ was not significant. The UV-VIS method had good intra-day repeatability (RSD from 0.104% to 0.239%) and inter-day repeatability (RSD = 0.24%). Under the conditions of this study the detection limit was $1.43 \cdot 10^{-3}$ mg·mL^{-1} of epirubicin and the quantitation limit was $4.34 \cdot 10^{-3}$ mg·mL^{-1} of epirubicine. Validation results indicated that this method may be used to

determine the photostability of epirubicin in aqueous solutions. The initial photodegradation study of epirubicin in solutions proved its photosensivity involving the use of a chemical actinometer. Epirubicin was almost completely degraded, by the time the absorbance of the quinine chemical actinometer decreased by the value of 0.2, which is significantly less than required 0.9. During this part of the study the colour of the epirubicin solution changed from red to colorless.

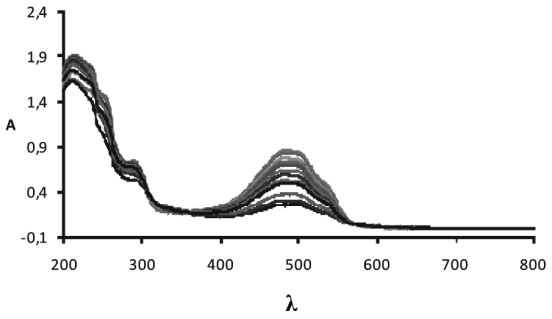

Fig. 1. Changes in UV spectra of water solution of epirubicin (c = 0.032 mg mL^{-1}) after different time of photodegradation; cylindrical quartz cell − optical pathlength 1 cm, λ = 365 nm, photodegradation time 545 min

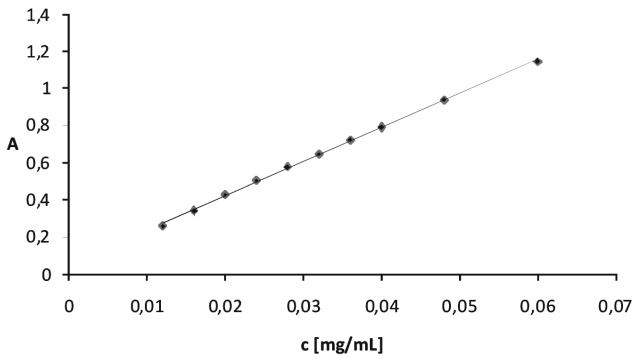

Fig. 2. The calibration curve of the determination of epirubicin

The photodegradation of epirubicin was a pseudo-first-order reaction. The absorbance of the epirubicine samples under radiation at λ_{max} = 365 nm decreased from A_{max} to $A_{\infty} > 0$ over a period of time from t_0 to t_{∞} (Fig. 3). The use of the subtraction technique showed that the dependence $\ln (A - A_{\infty}) = f(t)$ was linear (Fig. 4), so the degradation of epirubicin is described by the following equation: $\ln (A - A_{\infty}) = \ln (A_0 - A_{\infty}) - k_{obs} \cdot t$, where: A_0, A, A_{∞} – absorbance at time 0, t and ∞, respectively, k_{obs} – observed rate constant reaction of epirubicin degradation.

The rate constant of the reaction was equal to the slope of the plot of $\ln (A_t - A_{\infty}) = f(t)$, taken with the opposite sign and was $(1.30 \pm 0.39) \cdot 10^{-4}$ [s^{-1}]. During this part of the study the colour of the epirubicin solution changed from red to pale red.

The absorbance of the epirubicin samples under radiation at λ_{max} = 510 nm decreased from A_{max} to A = 0 over a period of time from t_0 to t_{∞} (Fig. 5). The dependence $\ln A = f(t)$ was linear (Fig. 6) and it is described by following equation: $\ln A = \ln A_0 - k_{obs} \cdot t$, where: A_0, A – absorbance at time 0, t and ∞, respectively, k_{obs} – observed rate constant reaction of epirubicin degradation.

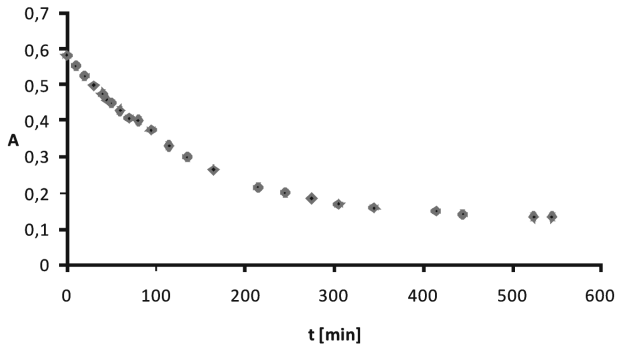

Fig. 3. Linear dependence A = f(t) of epirubicin photodegradation in solutions at isolated wavelength 365 nm region

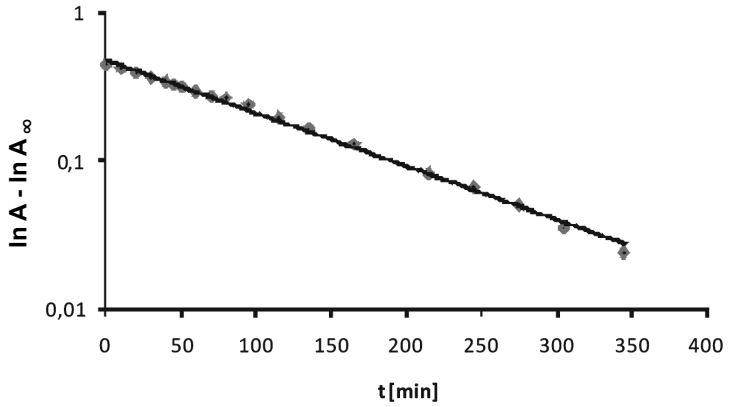

Fig. 4. Semilogarithmic plots for the photodegradation of epirubicin in solutions at isolated wavelength 365 nm region

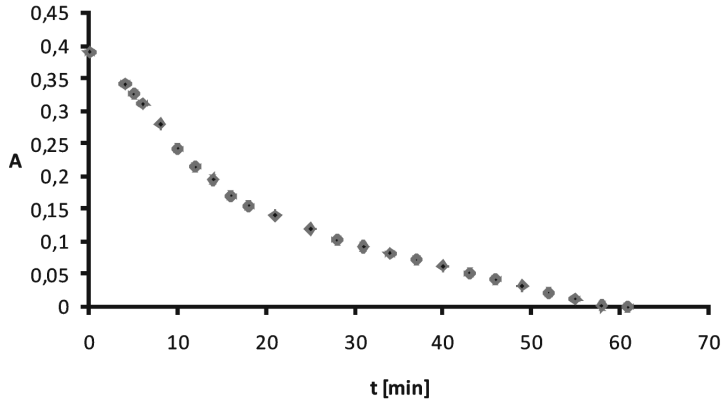

Fig. 5. Linear dependence A = f(t) of epirubicin photodegradation in solutions at isolated wavelength 510 nm region

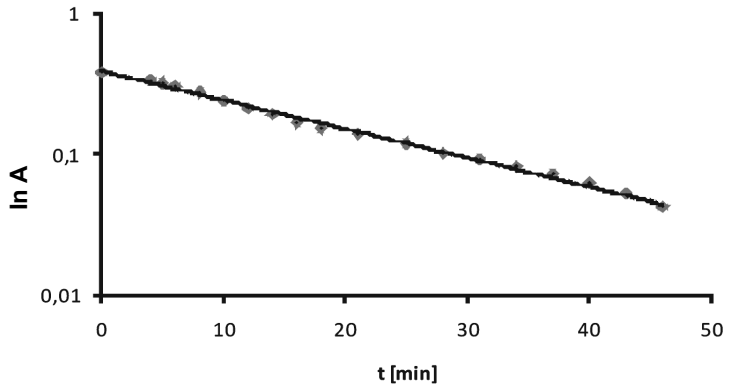

Fig. 6. Semilogarithmic plots for the photodegradation of epirubicin in solutions at isolated wavelength 510 nm region

The rate constant of the reaction was equal to the slope of the plot of ln A = f(t), taken with the opposite sign and was $(8.40 \pm 0.64) \cdot 10^{-4}$ [s^{-1}]. During this part of the study the colour of the epirubicin solution changed from red to colorless.

The degradation of epirubicin under radiation at λ_{max} = 510 nm is significantly faster and has a different mechanism compared to the degradation of epirubicin under radiation at λ_{max} = 365 nm. In order to determine changes in the kinetic parameters of the photochemical degradation of epirubicin quantum yields were calculated. The quantum yields for the photodegradation of epirubicin solutions upon exposure to radiation at λ_{max} = 365 nm and λ_{max} = 510 nm were $6.10 \cdot 10^{-5}$ and $1.26 \cdot 10^{-4}$, respectively.

CONCLUSIONS

The photodegradation of epirubicine in solution is a pseudo-first-order reaction which depends on substrate concentration. Results of the kinetic studies and quantum yields suggest that a water solution of epirubicin is more susceptible to degradation upon exposure to light with λ_{max} = 365 nm than λ_{max} = 510 nm.

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SUMMARY

Photostability of epirubicin – an anticancer drug – was investigated. The solution samples were exposed to UV-A and VIS radiation and the photodegradation process was monitored by using a spectrophotometric method. Epirubicin was shown to be photolabile and its photodegradation was a first-order reaction. The quantum yields of each photodegradation process were also calculated.

STRESZCZENIE

Zbadano fototrwałość epirubicyny – leku przeciwnowotworowego. Roztwory badane poddano ekspozycji na promieniowanie UV-A i VIS, a proces fotodegradacji analizowano metodą spektrofotometryczną. Epirubicyna była fotolabilna, a jej fotodegradacja zachodziła zgodnie z kinetyką pierwszego rzędu. Obliczono także wydajności kwantowe każdej reakcji fotodegradacji.