

Photo-Stability of the New Antiplatelet Agent, KBT-3022 (Ethyl 2-[4,5-Bis(4-methoxyphenyl)thiazole-2-yl]pyrrol-1-ylacetate) in Aqueous Solutions Containing Acetonitrile

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The photo-stability of ethyl 2-[4,5-bis(4-methoxyphenyl)thiazole-2-yl]pyrrol-1-ylacetate, KBT-3022 in aqueous solutions containing acetonitrile was investigated under the light of a high pressure mercury lamp. Its main photo-degradation product was assumed to be ethyl 5-hydroxy-5-[4,5-bis(4-methoxyphenyl)thiazole-2-yl]-2-oxo-3-pyrrolin-1-ylacetate. KBT-3022 was also found to undergo ester hydrolysis by heat in both acidic and basic aqueous solutions, but its hydrolysis was confirmed to be negligible in the range of pH 3—9 at room temperature (25°C). Further, its photo-stability with exposure to the high pressure mercury lamp was comparable to that with exposure to a fluorescent lamp. Therefore, it is considered feasible to simulate the photo-stability of KBT-3022 in aqueous solutions containing acetonitrile by exposure to all other light sources including diffuse daylight, if the cumulative number of photons of the light can be determined by actinometry.

Keywords photo-stability; accelerated test; KBT-3022; photodegradation; hydrolysis; high pressure mercury lamp

There are many reports relating to the degradation kinetics of drugs which undergo photodegradation in aqueous solutions. In the case of drugs being stable by heat, it is possible to estimate the photo-stability of the drugs in a given normal conditions from a photo-stability test under an appropriate light source for an accelerated test.¹⁾ However, in the case of drugs being unstable in the dark by heat, no method for predicting the photo-stability of the drugs has yet been established.

Ethyl 2-[4,5-bis(4-methoxyphenyl)thiazole-2-yl]pyrrol-1-ylacetate, KBT-3022, a platelet aggregation inhibitor, has a chemical structure of substituted diphenylthiazole, as shown in Chart 1.^{2,3)} KBT-3022 has been already found to be photo-labile in aqueous solutions containing acetonitrile when exposed to artificial light from our preliminary experiments. KBT-3022 also undergoes ester hydrolysis in the solutions by heat. Therefore, it is important to confirm quantitatively the stability of KBT-3022 in the dark as a control to predict its photo-stability.

On the other hand, the new Japanese guidelines for

“stability studies” were made public in February, 1991. These guidelines also provide the standard methods to conduct photo-stability tests. We thought that it was necessary to establish an accelerated photo-stability test in order to predict the photo-stability of drugs in a short period. In this case, it is very important to select a suitable light source for the accelerated test. That is to say, the selected light source must have enough wavelength intensity to contribute to the photodegradation of drugs.

In this work, we quantitatively investigated a variety of factors influencing the stability of KBT-3022 in order to predict its photo-stability under optional environments.

Experimental

Materials KBT-3022 was prepared in our laboratory and used without further purification. All other chemicals were of reagent grade, and distilled water was taken from a Milli-QII water purification system (Millipore Ltd.).

Hydrolysis Kinetic Procedure Since KBT-3022 is water-insoluble, an aqueous buffer solution containing 33.3, 50 and 66.7% (v/v) acetonitrile was used as a reaction medium. The ionic strength of all the used buffer

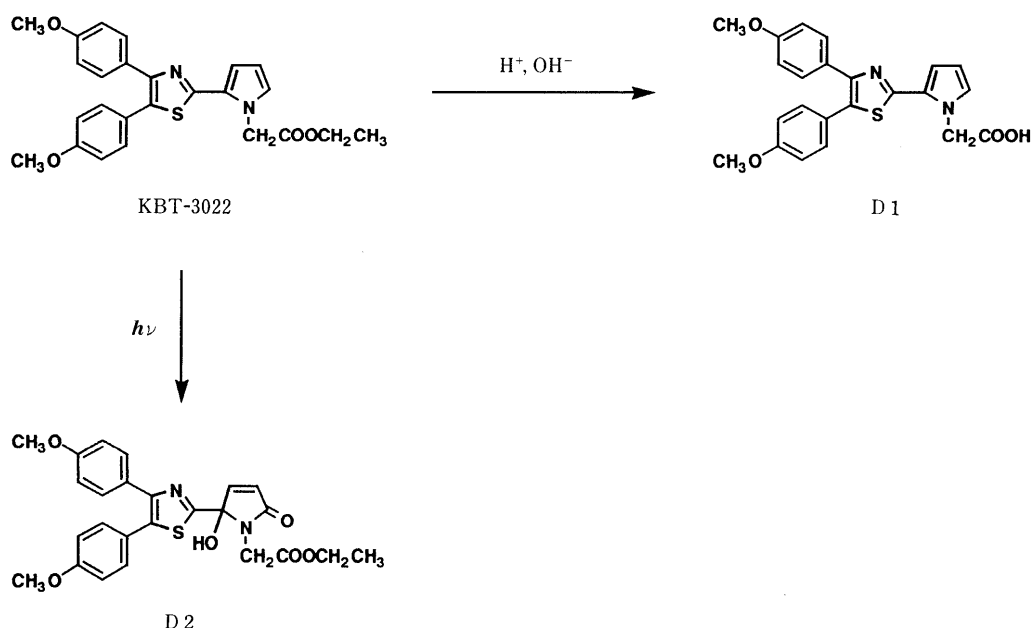


Chart 1. Pathway for the Hydrolysis and the Photodegradation of KBT-3022 in Aqueous Solutions Containing Acetonitrile

solution was adjusted to 0.1. Brown colored glass ampules 10 ml in volume containing 3 ml of the sample solution were enclosed and covered with aluminum foil for light protection, and then the ampules were placed in a preheated air-circulation oven at 60, 70 and $80 \pm 1^\circ\text{C}$.

Photodegradation Kinetic Procedure The photodegradation kinetics of KBT-3022 were studied in an aqueous unbuffered solution containing 50% (v/v) acetonitrile, at room temperature. As shown in previous reports,^{1,4)} an exposure test was performed with a merry-go-round type apparatus. In the case of a high pressure mercury lamp (300 W) being used as light source, the distance between the lamp and Pyrex glass tube (15 ml) with a stopper containing the sample solution (15 ml) was approximately 120 mm. A UV-D33S colored glass filter (Toshiba Garasu Kogyo Co., Ltd.)¹⁾ was attached at a window of the high pressure mercury lamp. All parts other than the window were covered with aluminum foil. In the case of irradiation by a fluorescent lamp (15W \times 2), a test box¹⁾ with an attached UV-D33S colored glass filter was used. The distance between the fluorescent lamp and Pyrex glass tube (15 ml) with a stopper was 300 mm. For measurement of light intensity, a ferrioxalate actinometer was used.^{1,4)} In the case using the high pressure mercury lamp, the light quanta was 1.5×10^{16} quanta/s and in the case using the fluorescent lamp, it was 1.3×10^{14} quanta/s.

Assay of Intact KBT-3022 For the determination of intact KBT-3022, 2.0 ml of the sample solution and 1.0–2.0 ml of the internal standard (phenanthrene 5–10 $\mu\text{g}/\text{ml}$ in acetonitrile) were diluted exactly to make 20 ml with the mobile phase of high performance liquid chromatography (HPLC), and then injected at room temperature.

The HPLC system consisted of a Shimadzu model LC-5A pump, an SPD-2A UV-detector, a Chromatopack C-R4A integrator, a Reodyne 7125 injector with a 20 μl loop and a Unisil pack 5C18-150A column (4.6 i.d. \times 150 mm, particle size 5 μm , GL Sciences Inc.). The mobile phase used for the determination of KBT-3022 was acetonitrile–0.01 M potassium dihydrogen phosphate (adjusted to pH 3.5 with phosphoric acid) (80:20). The flow rate was 0.8 ml/min and the detection of the column eluate was monitored at 254 nm.

Isolation and Identification of Main Degradation Products of KBT-3022

1) Hydrolysis Product of KBT-3022 Fifty milligrams of KBT-3022 were dissolved in 10 ml of acetonitrile, and then a mixture of 0.1 N HCl–acetonitrile (1:1) or 0.1 N NaOH–acetonitrile (1:1) was added to make 50 ml. Each solution was refluxed at 50°C on a water bath for 5 h and 39 h, respectively, with protection from light. After neutralization of the degraded solution under a basic condition, the solution was evaporated and extracted three times with ethyl acetate. The ethyl acetate solution was washed twice with water, dried over anhydrous sodium sulfate, and concentrated under reduced pressure to afford a pale green crystalline solid. The degraded solution under an acidic condition was diluted and subjected to HPLC.

2) Photodegradation Product of KBT-3022 One gram of KBT-3022 was dissolved in 100 ml of acetonitrile, and then 900 ml of a mixture of H_2O –acetonitrile (1:1) was added. The solution was irradiated under the high pressure mercury lamp without a UV-D33S colored glass filter at room temperature for 30 h. The photodegraded solution was evaporated, and then the residual solution was extracted with ethyl acetate, and concentrated under reduced pressure. The oily residue was charged twice on a preparative thin layer chromatography (TLC) plate (Kieselgel 60 F_{254S}, Merck) using CHCl_3 –MeOH (20:1) as the developing solvent. The band detected at 4.7 min of the retention time on HPLC (column: 5C18-200A, 4.6 i.d. \times 200 mm, particle size 5 μm , GL Sciences Inc.; the mobile phase: acetonitrile–0.01 M potassium dihydrogen phosphate adjusted to pH 3.5 with phosphoric acid (70:30); the flow rate: 1.0 ml/min) was the main photodegradation product. It was extracted with ethyl acetate and concentrated under reduced pressure to afford an oily product. The crude product was further treated on a preparative TLC plate using ethyl acetate as the developing solvent. The main band was extracted with ethyl acetate, concentrated under reduced pressure, and crystallized with petroleum ether to give an orange crystalline solid, mp 67 – 71°C . ¹H-NMR (CDCl_3) δ : 1.26 (3H, t, $J=7.1$ Hz, CH_2 – CH_3), 3.79, 3.82 (6H, two s, O– CH_3), 3.92, 4.56 (2H, d, $J=18.0$ Hz, N– CH_2), 4.15–4.23 (2H, m, CH_2 – CH_3), 5.21 (1H, s, OH), 6.29, 7.18 (2H, two d, $J=5.9$ Hz, (CH=CH) 6.80–7.41 (8H, m, arom.). MS m/z : 480 (M^+).

Results and Discussion

Isolation and Identification of the Main Degradation Products of KBT-3022

1) Hydrolysis Product of KBT-3022 Under a basic condition, KBT-3022 was heated in

acetonitrile–0.1 N NaOH (60:40) at 50°C for 5 h with protection from light. The retention time of the isolated basic-catalyzed degradation product on HPLC was identical with that of the authentic sample of desethyl KBT-3022 (D1) which was separately synthesized. Further, the infrared (IR) spectrum of the degradation product also agreed with that of desethyl KBT-3022. On the other hand, under an acidic condition, KBT-3022 was heated in acetonitrile–0.1 N HCl (60:40) at 50°C for 39 h in the dark. The degraded solution gave a single peak other than that of KBT-3022 on HPLC. The retention time of the peak was also identical with that of the authentic sample of desethyl KBT-3022. Therefore, KBT-3022 was found to undergo ester hydrolysis under both acidic and basic conditions.

2) Photodegradation Product of KBT-3022 KBT-3022 was exposed to the high pressure mercury lamp in acetonitrile– H_2O (55:45) for 30 h. The photodegraded solution was analyzed on HPLC. Its main photodegradation detected at 4.7 min of the retention time on HPLC was identified by proton nuclear magnetic resonance (¹H-NMR), carbon-13 nuclear magnetic resonance (¹³C-NMR), IR and mass spectrum (MS). In the ¹H-NMR spectrum, the signal of the proton at the C-5 position on a pyrrole ring was not detected, but the signal of the hydroxy proton which was exchangeable with CD_3OD was detected. Further, the signals of two carbonyl carbons were detected in the IR spectrum, and the ¹³C-NMR spectrum supported the presence of two carbonyl carbons. Since these results indicated the formation of hydroxylactam, the photodegradation product was assumed to be ethyl 5-hydroxy-5-[4,5-bis(4-methoxyphenyl)thiazole-2-yl]-2-oxo-3-pyrrolin-1-ylacetate (D2), as shown in Chart 1. The compound was probably formed as the result of sensitized photooxidation of KBT-3022. Studies of the detailed mechanism of the photodegradation of KBT-3022 are in progress.

Hydrolysis Kinetics of KBT-3022 Since KBT-3022 is hydrolyzed to afford desethyl KBT-3022 (D1) in aqueous solutions containing acetonitrile, it is important to confirm quantitatively the stability of KBT-3022 in the dark as a control to predict its photo-stability. Thus, the hydrolysis kinetics of KBT-3022 were studied in aqueous buffer (pH 3–11) solutions containing 33.3, 50 and 66.7% (v/v) acetonitrile at elevated temperatures with protection from light. The ester hydrolysis of KBT-3022 was found to be simulated by pseudo-first-order kinetics. The pseudo-first-order rate constants (k_{hyd}) for the hydrolysis of KBT-3022 were calculated by the least-square linear regression method.

1) Effect of pH The effect of pH on the hydrolysis of KBT-3022 was investigated in an aqueous buffer solution containing 50% acetonitrile at 60°C . As shown in Fig. 1, the hydrolysis rate of KBT-3022 increased with an increasing hydrogen ion concentration of the aqueous buffer solution in the range of pH 3–4, and an increasing hydroxide ion concentration in the range of pH 4–11. KBT-3022 was found to be most stable at approximately pH 4. The half-lives varied from 11 h to 1853 d for the hydrolysis of KBT-3022 within the pH range of 3–11 at 60°C .

2) Effect of Temperature It is necessary to confirm the stability of KBT-3022 at room temperature (25°C) in order to predict its photo-stability precisely. An Arrhenius plot

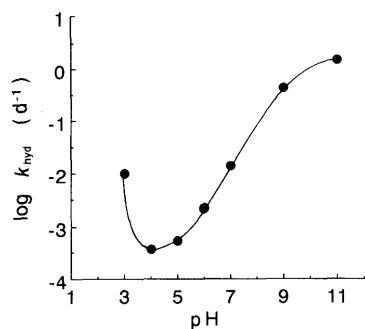


Fig. 1. The pH-Rate Profile for the Hydrolysis of KBT-3022 in Aqueous Solutions Containing 50% (v/v) Acetonitrile at 60 °C

Initial concentration: 100 μ g/ml.

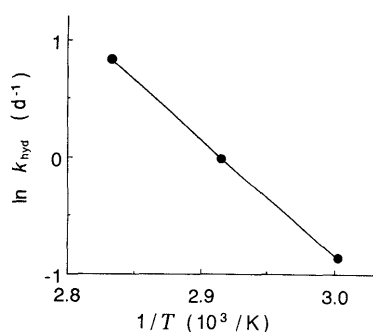


Fig. 2. Arrhenius Plot for the Hydrolysis of KBT-3022 in Aqueous Solutions Containing 50% (v/v) Acetonitrile at pH 9

Initial concentration: 100 μ g/ml.

being linear with a correlation coefficient of $r=0.999$ at pH 9 was given in Fig. 2. The activation energy for the hydrolysis of KBT-3022 in an aqueous pH 9 buffer solution containing 50% acetonitrile in the range of 60–80 °C was 19.6 kcal/mol. The 10% loss time ($T_{90\%}$) of KBT-3022 was estimated to be approximately 7.7 d at pH 9 and 25 °C from the Arrhenius plot. Therefore, the hydrolysis of KBT-3022 seemed to be almost negligible in the range of pH 3–9 at room temperature.

3) Effect of Acetonitrile Content in the Reaction Medium

Since KBT-3022 is water-insoluble, the drugs must be solubilized in an aqueous environment free from any organic solvent of the gastrointestinal tract in order to be absorbed. Therefore, it is important to simulate the stability of KBT-3022 in an aqueous solution free from acetonitrile. The effect of the acetonitrile content in the reaction medium on the hydrolysis of KBT-3022 was examined at 60 °C. The aqueous buffer (pH 9, 11) solution/acetonitrile (v/v) ratios were 2 : 1, 1 : 1, 1 : 2, respectively. As the acetonitrile content in the reaction medium decreased, the rate constant of its hydrolysis increased. This result is assumed to be due to an alteration of the polarity of the medium which affects the transition state of the hydrolysis of KBT-3022. That is to say, since the dielectric constant increases with decreasing acetonitrile content, KBT-3022 appears to become more unstable.

As shown in Fig. 3, the logarithm of the rate constant for the hydrolysis of KBT-3022 was found to be linearly related to the acetonitrile concentration with correlation coefficients of $r=0.999$ at pH 9 and $r=0.998$ at pH 11, respectively. Therefore, it will be possible to extrapolate the

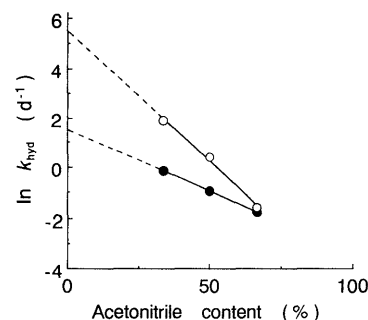


Fig. 3. Effect of Acetonitrile Content on the Hydrolysis of KBT-3022 in Aqueous Solutions at 60 °C

Initial concentration: 10 μ g/ml. ●, pH 9; ○, pH 11.

stability of KBT-3022 in an aqueous buffer solution containing various amounts of acetonitrile from these approximate equations. For instance, a half-life of KBT-3022 in an aqueous pH 9 buffer solution free from acetonitrile was simulated to be approximately 3.6 h at 60 °C. That is to say, KBT-3022 was assumed to be very unstable in aqueous solutions, a finding which was also supported by administration of KBT-3022 *in vivo*. After oral administration to rats, intact KBT-3022 was not detected in plasma, but desethyl KBT-3022 was detected, although most of the KBT-3022 was hydrolyzed by esterase *in vivo*.⁵⁾ In addition, if this procedure was used for not only KBT-3022 but other water-insoluble drugs, its stability in aqueous solutions free from any organic solvent may be simulated.

Photodegradation Kinetics of KBT-3022 KBT-3022 is not only hydrolyzed but also photo-labile in solutions. When exposed to artificial light, KBT-3022 in an aqueous solution containing acetonitrile easily undergoes photodegradation to afford D2 as its main photodegradation product.

KBT-3022 in a 50% (v/v) acetonitrile solution shows an absorbance maxima at approximately 345 nm which belongs to the near ultraviolet (UV) region, probably contributing to its photodegradation. The high pressure mercury lamp has a strong intensity in the near UV region compared with the UV and visible regions. Thus, we selected the high pressure mercury lamp as a light source for the accelerated test, because it will be possible that the photo-stability of KBT-3022 under a given intensity of all other light sources can be predicted in a short period.

In this photo-stability study, we have used ferrioxalate actinometer to determine the cumulative number of photons as an index of light intensity.^{1,4)} Further, a UV-D33S colored glass filter which protects the visible light in the wavelength range over 420 nm was used,¹⁾ since the ferrioxalate actinometer shows a constant quantum yield in the wavelength range of 250 to 420 nm.

1) Effect of KBT-3022 Initial Concentration In general, the rate of photodegradation of drugs in solutions has been exhibited apparent first-order kinetics, for example, adriamycin,⁶⁾ furosemide,⁷⁾ ketrolac trometamine,⁸⁾ and theophylline.⁹⁾ Further, Connors *et al.* reported¹⁰⁾ that the rate of photodegradation of a drug, or the rate of product formation, follows approximate first-order kinetics in a low initial concentration, but it obeys pseudo-zero-order kinetics in a high initial concentration. The reason for

TABLE I. Apparent First-Order Kinetic Data for the Photodegradation of KBT-3022 as a Function of Initial Concentration

Initial conc. ($\mu\text{g/ml}$)	$k_{\text{pho}}^{a,b}$ ($\text{quanta}^{-1} \times 10^{20}$)	Half-life ^{c)} ($\text{quanta} \times 10^{-19}$)	Correlation coefficient
10	6.32	1.10	0.997
20	7.17	0.967	0.998
50	6.91	1.00	0.999
100	5.49	1.26	0.999
200	3.63	1.91	0.998

a) The apparent first-order rate constants for photodegradation of KBT-3022 in 50% (v/v) acetonitrile solutions exposed to the high pressure mercury lamp passed through UV-D33S colored glass filter. b) Light quanta that indicates the intensity of light was 1.5×10^{16} quanta/s. c) The cumulative number of photons which 50% of KBT-3022 photodegradates.

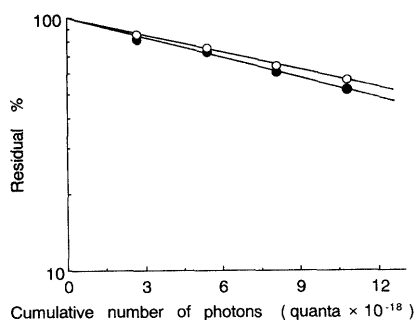


Fig. 4. Effect of pH on the Photodegradation of KBT-3022 in Aqueous Solutions Containing 50% (v/v) Acetonitrile under the High Pressure Mercury Lamp

Initial concentration: 100 $\mu\text{g/ml}$. ●, pH 3, 5, 7; ○, pH 9.

the change in reaction order was stated as follows. The reaction becomes limited by the number of incident quanta of energy, and in concentrated solutions, quenching of the excited molecules becomes more efficient.

In the case of KBT-3022, plots of the logarithm of intact KBT-3022 (%) versus the cumulative number of photons showed a linear relationship in the initial concentration range of 10–200 $\mu\text{g/ml}$. Thus, the apparent first-order rate constants (k_{pho}) for the photodegradation of KBT-3022 were estimated from the least-square linear regression method.

The apparent first-order rate constants were nearly constant in the range of 10 to 50 $\mu\text{g/ml}$, as shown in Table I. In other words, in this concentration range, the half-lives for photodegradation were independent of the initial concentration. On the other hand, in concentrations over 100 $\mu\text{g/ml}$, the apparent first-order rate constant decreased with as the initial concentration of KBT-3022 increased, indicating that the quenching of excited KBT-3022 occurred more frequently, as reported by Connors *et al.*

2) Effect of pH The effect of pH on the photodegradation of KBT-3022 was examined (Fig. 4). Although the hydrolysis rate of KBT-3022 was dependent on the pH of the aqueous solution as mentioned above, its photodegradation rate was not highly affected by pH. This result demonstrated that the pH of an aqueous solution influenced only the hydrolysis of KBT-3022.

3) Effect of Light Source The high pressure mercury lamp used in this work is vigorously different from a fluorescent lamp in the distribution of spectral radiation energy. The fluorescent lamp mainly irradiates visible light compared with near UV light. Thus, the effect of the light source on the photodegradation of KBT-3022 was investigated. Since a UV-D33S colored glass filter was also

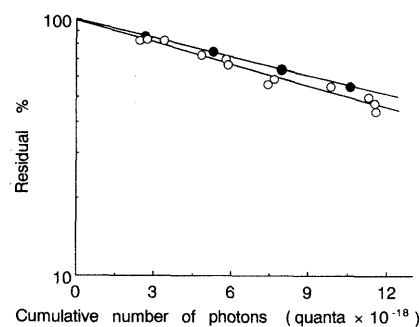


Fig. 5. Effect of Light Source on the Photodegradation of KBT-3022 in Aqueous Solutions Containing 50% (v/v) Acetonitrile

Initial concentration: 100 $\mu\text{g/ml}$. ●, high pressure mercury lamp; ○, fluorescent lamp.

attached when KBT-3022 was exposed to the fluorescent lamp, the visible light in the wavelength range over 420 nm could not pass through it. Figure 5 showed the photo-stability of KBT-3022 under light from two light sources. The apparent first-order rate constant for its photodegradation with exposure to the fluorescent lamp agreed well with that from exposure to the high pressure mercury lamp. From this result, it is considered feasible to simulate the photo-stability of KBT-3022 under exposure to all other light sources, including diffuse daylight, if the cumulative number of photons of light passed through a UV-D33S colored glass filter can be determined by actinometry.

In conclusion, we have collected the following data for the hydrolysis of KBT-3022 in aqueous solutions containing acetonitrile. (1) KBT-3022 was most stable at pH 4. (2) The activation energy was 19.6 kcal/mol at pH 9. (3) KBT-3022 was more unstable as the acetonitrile content decreased. Then the photo-stability of KBT-3022 was investigated. (4) In the initial concentration below 50 $\mu\text{g/ml}$, the half-lives for the photodegradation of KBT-3022 were approximately constant. (5) Its photodegradation rate was not highly affected by pH. (6) Its photo-stability with exposure to the high pressure mercury lamp used as a light source for accelerated test agreed well with that from exposure to the fluorescent lamp.

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